



Sabu Thomas
Ajitha AR
Cintil Jose Chirayil
Bejoy Thomas
Editors

Handbook of Biopolymers

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Sabu Thomas • Ajitha AR •
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Handbook of Biopolymers

With 376 Figures and 55 Tables

 Springer

Editors

Sabu Thomas
School of Chemical Science
Mahatma Gandhi University
Kottayam, India

Ajitha AR
Mahatma Gandhi University
Kottayam, India

Cintil Jose Chirayil
Department of Chemistry
Newman College
Thodupuzha, India

Bejoy Thomas
Department of Chemistry
Newman College
Thodupuzha, India

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Preface

Biopolymers constitute some of the most diverse groups of organic molecules, which recently become an area of great research interest for their applications toward many dimensions of human life. Topics covered in this book include biopolymers from renewable sources, marine prokaryotes, soy protein and humus oils, biopolymer recycling, chemical modifications, and specific properties. Applications in key areas such as material, biomedical, sensing, packaging, biotechnology, and tissue engineering are discussed in detail. This handbook will be a very valuable reference material for graduate and postgraduate students, academic researchers, professionals, research scholars, scientists, and for anyone who has a flavor for doing biomaterial research. It provides universal perspectives for an emerging field where classical polymer science blends with molecular biology with highlights on recent advances.

Kottayam, India
Kottayam, India
Thodupuzha, India
Thodupuzha, India
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Sabu Thomas
Ajitha AR
Cintil Jose Chirayil
Bejoy Thomas

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About the Editors



Sabu Thomas Ph.D., FRSC (UK), D.Sc. (UL, France), D.Sc. (UBS, France)

Vice Chancellor, Mahatma Gandhi University, Priyadarshini Hills, Kottayam, Kerala, India

Professor, School of Chemical Sciences & Director, School of Energy Materilas

Founder Director, International and Inter University Centre for Nanoscience and Nanotechnology, Mahatma Gandhi University, Kottayam, Kerala, India

Professor Sabu Thomas is currently Vice Chancellor of Mahatma Gandhi University. He is also Full Professor of Polymer Science and Engineering at the School of Chemical Sciences of Mahatma Gandhi University, Kottayam, Kerala, India, and the Founder Director and Professor of the International and Interuniversity Centre for Nanoscience and Nanotechnology. Prof. Thomas is an outstanding leader with sustained international acclaim for his work in Nanoscience, Polymer Science and Engineering, Polymer Nanocomposites, Elastomers, Polymer Blends, Interpenetrating Polymer Networks, Polymer Membranes, Green Composites and Nanocomposites, Nanomedicine, and Green Nanotechnology. Prof. Thomas's groundbreaking inventions in polymer nanocomposites, polymer blends, green bionanotechnological, and nano-biomedical sciences have made transformative differences in the development of new materials for automotive, space, housing, and biomedical fields. In collaboration with India's premier tyre company, Apollo Tyres, Professor Thomas's group invented new high-performance barrier rubber nanocomposite membranes for inner tubes and inner liners for tyres. Professor Thomas has received a

number of national and international awards which include: Fellowship of the Royal Society of Chemistry, London FRSC, Distinguished Professorship from Josef Stefan Institute, Slovenia, MRSI medal, Nano Tech Medal, CRSI medal, Distinguished Faculty Award, Dr. APJ Abdul Kalam Award for Scientific Excellence – 2016, Mahatma Gandhi University – Award for Outstanding Contribution – Nov. 2016, Lifetime Achievement Award of the Malaysian Polymer Group, Indian Nano Biologists award 2017, and Sukumar Maiti Award for the best polymer researcher in the country. He is on the list of most productive researchers in India and holds a position of No. 5. Because of his outstanding contributions to the field of Nanoscience and Polymer Science and Engineering, Prof. Thomas has been conferred HonorisCausa (D.Sc.) Doctorate by the University of South Brittany, Lorient, France and the University of Lorraine, Nancy, France. Very recently, Prof. Thomas has been awarded Senior Fulbright Fellowship to visit 20 universities in the United States and most productive faculty award in the domain Materials Sciences. Very recently he was also awarded with National Education Leadership Award – 2017 for Excellence in Education. Prof. Thomas also won 6th contest of “mega-grants” in the grant competition of the Government of the Russian Federation (Ministry of Education and Science of the Russian Federation) designed to support research projects implemented under the supervision of the world’s leading scientists. He has been honored with Faculty Research Award of India’s brightest minds in the field of academic research in May 2018. Professor Thomas was awarded with Trila – Academician of the Year in June 2018 acknowledging his contribution to tyre industry. In 2019 Professor Thomas has been selected as a member of Prestigious European Academy of Sciences. Last year he was honored with C.N.R. Rao Award Prize Lecture in Advanced Materials by Materials Research Society of India (MRSI). In 2019, Professor Thomas has been selected as a Member of Prestigious European Academy of Sciences. He was also selected as the Fellow of International Academy of Physical Sciences and was honored with Life Time Achievement award by the Indian Association of Solid State Chemists and Allied Scientists (ISCAS). This year he was selected for the DST Nanomission award and very recently in May

25, 2020, he was selected for the honorary title of “Honoured Professor of Siberian Federal University.” Professor Thomas has published over 1000 peer-reviewed research papers, reviews, and book chapters. He has co-edited and written 137 books published by Royal Society, Wiley, Woodhead, Elsevier, CRC Press, Springer, Nova, etc. He is the inventor of 15 patents. Prof. Thomas has guided nearly 107 Ph.D. scholars in India and abroad. The H index of Prof. Thomas is 101 and has more than 50356 citations. Prof. Thomas has delivered over 350 plenary/inaugural and invited lectures in national/international meetings in over 30 countries.



A. R. Ajitha is Senior Researcher at International and Interuniversity Centre for Nanoscience and Nanotechnology (IIUCNN), Mahatma Gandhi University. She completed her doctoral research in the area of Polymer Science, incorporating nanomaterials in thermoplastics. Her research area includes preparation of nanocomposites, their characterization, and modification of nanofillers. She received 2 patents and published 11 research articles in international journals of high impact factor. She edited one book titled *Compatibilization of Polymer Blends* (Elsevier publication). She has also authored more than 14 book chapters in international books and presented papers at many international conferences and seminars. She is an expert in the field of engineering application of nanomaterials.



Cintil Jose Chirayil is Assistant Professor at the Department of Chemistry, Newman College, Kerala, India. She received her Ph.D. in Polymer Science from Mahatma Gandhi University, India, and completed a post-doctoral fellowship at the Centre for Advanced Materials, Qatar University, Doha. She has 12 years of research experience in the field of nanoscience and nanocellulose with 20 publications in international peer-reviewed journals and 15 book chapters and 1 edited book with Elsevier International publishing company. In addition to the Young Scientist Award, 2011 (Leed's University London and NIT Calicut), she was the recipient of Foreign scholarships from the

University of Potsdam, Germany; University of Mara, Malaysia; and Institute of Chemistry, Austria, for in-depth research in the field of nanoscience. She is an approved research guide of Mahatma Gandhi University, Kottayam, in the subject area Chemical Sciences.



Bejoy Thomas studied Chemistry at the University of Bangalore (India) and received his Ph.D. in Physical Chemistry from Cochin University of Science and Technology (India). As a DFG (German Chemical Society) Postdoctoral Fellow at the Institute of Chemical Technology, University of Stuttgart, he moved to Germany after working for a few years in NMR spectroscopy at the NMR Research Centre at the Indian Institute of Science (India) under CSR-SRF. Before returning to India to join Newman College in Thodupuzha, Kerala, as Assistant Professor of Chemistry in 2011, Dr. Bejoy first moved to Paris, France, as a CNRS Postdoctoral Associate at the Laboratoire de Chimie de la Matière de Condensée de Paris (LCMCP), UPMC-P6-Paris. His research focuses on the self-assembly of biosurfactant molecules, such as long-chain fatty acids and their esters, small proteins, polysaccharides, etc. for the development of new materials, the creation of organized mesoporous materials, surface science, catalysis, and hierarchical zeolites, as well as in situ NMR spectroscopy of working catalysts. The extraction, properties, and use of nanocellulosic materials from biomass resources have recently been the subject of active research, particularly with regard to catalytic applications. Nearly 50 research articles by Dr. Bejoy have been published in high impact journals, including one in *Chemical Reviews* that has received the highest citations in recent years from authors in India.

Contributors

Rashid Sulthan A. Department of Chemistry, Amrita Vishwa Vidyapeetham, Amritapuri, Kollam, Kerala, India

Saithalavi Anas Advanced Molecular Materials Research Centre, Mahatma Gandhi University, Kottayam, India

V. N. Anjana Department of Chemistry, Sree Sankara Vidyapeetom College, Valayanchirangara, Kerala, India

T. R. Anju Department of Biotechnology, Newman College, Thodupuzha, Kerala, India

Sherin Antony Department of Zoology, Bharata Mata College, Thrikkakara, Kerala, India

Shinar Athwal University Institute of Biotechnology, Chandigarh University, Mohali, Punjab, India

Monika Awana Division of Biochemistry, ICAR – Indian Agricultural Research Institute (IARI), New Delhi, India

Preetha Balakrishnan Centre for Innovations and Technologies (CIT), ADSO Naturals Private Limited, Bangalore, India

Andrea Cristiane Krause Bierhalz Department of Textile Engineering, Federal University of Santa Catarina-Campus Blumenau, Blumenau, SC, Brazil

Muhammad Bilal Institute of Chemical Technology and Engineering, Faculty of Chemical Technology, Poznan University of Technology, Poznan, Poland

T. K. Bindu Sharmila Department of Chemistry, Maharaja's College, Ernakulam, India

J. S. Binoj Institute of Mechanical Engineering, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India

Srinivasa Budagumpi Centre for Nano and Material Sciences, Jain University, Bangalore, India

Sreelekshmi C. Department of Chemistry, Amrita Vishwa Vidyapeetham, Amritapuri, Kollam, Kerala, India

Ashwini Chauhan Department of Microbiology, Tripura University (A Central University), Agartala, Tripura, India

Suchitra Rajput Chauhan Department of Applied Science, School of Engineering and Technology, BML Munjal University, Gurugram, Haryana, India

Reeba Mary Cherian Department of Chemistry, Newman College, Thodupuzha, Kerala, India

School of Chemical Sciences, Mahatma Gandhi University, Kottayam, Kerala, India

Cintil Jose Chirayil Department of Chemistry, Newman College, Thodupuzha, India

Ji-hyun Cho Laboratory of Adhesion & Bio-Composites, Program in Environmental Materials Science, Seoul National University, Seoul, Republic of Korea

Mariana Altenhofen da Silva Center of Agricultural Sciences, Federal University of São Carlos, Araras, SP, Brazil

Pragnesh Dave Department of Chemistry, Sardar Patel University, Gujarat, India

R. Divya Department of Electronics and Communication Engineering, Sree Narayana Institute of Technology, Adoor, Affiliated to A P J Abdul Kalam Technological University Kerala, Thiruvananthapuram, India

Samuel Ogbeide Ebhodaghe Chemical Engineering Department, University of Benin, Benin City, Edo State, Nigeria

Lekshmi K. Edison Department of Comparative, Diagnostic and Population Medicine, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

Meenu Eldhose Department of Chemistry, Newman College, Thodupuzha, India

K. S. Femina Post-Graduate and Research Department of Chemistry, Maharajas College, Ernakulam, India

Marta Fernandes Centre for Textile Science and Technology (2C2T), University of Minho, Guimarães, Portugal

Rui D. V. Fernandes Centre for Textile Science and Technology (2C2T), University of Minho, Guimarães, Portugal

Ashoka Gamage Department of Chemical and Process Engineering, Faculty of Engineering, University of Peradeniya, Peradeniya, Sri Lanka

Benny George Rubber Technology Division, Rubber Research Institute of India, Rubber Board, Kottayam, India

Cincy George Department of Chemistry, Newman College, Thodupuzha, India

Liz George Department of Chemistry, Newman College/Nirmala College, Thodupuzha, India

Neena George Post Graduate and Research Department of Chemistry, Maharaja's College, Ernakulam, Kerala, India

M. T. Giffin MG University (MGU), Kottayam, India

Geethy P. Gopalan Advanced Molecular Materials Research Centre, Mahatma Gandhi University, Kottayam, India

Sreerag Gopi Centre for Innovations and Technologies (CIT), ADSO Naturals Private Limited, Bangalore, India

Arun Gupta Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang, Gambang, Pahang, Malaysia

Shreya Gupta University Institute of Biotechnology, Chandigarh University, Mohali, Punjab, India

Mateen Hedar School of Chemistry, University of the Punjab, Lahore, Pakistan

Mohamed Saad Bala Husain Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang, Gambang, Pahang, Malaysia

Nazim Hussain Center for Applied Molecular Biology (CAMB), University of the Punjab, Lahore, Pakistan

Nazim Hussain Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan

Tajamal Hussain School of Chemistry, University of the Punjab, Lahore, Pakistan

Azeem Intisar School of Chemistry, University of the Punjab, Lahore, Pakistan

Albin James Mahatma Gandhi University Centre for Research in Chemistry, Newman College, Thodupuzha, Kerala, India

Mahatma Gandhi Centre for Teacher Education, Thodupuzha, Kerala, India

Hyeon Su Jo Laboratory of Adhesion & Bio-Composites, Program in Environmental Materials Science, Seoul National University, Seoul, Republic of Korea

Sona John Department of Chemistry, Newman College, Thodupuzha, India

Cintil Jose Department of Chemistry, Newman College, Thodupuzha, Kerala, India

Alex Joseph Department of Chemistry, Newman College, Thodupuzha, India
Department of Chemistry, Nirmala College, Muvattupuzha, India

Anu Mary Joseph Department of Chemistry, Newman College, Thodupuzha, India

C. S. Julie Chandra Department of Chemistry, Maharaja's College, Ernakulam, India

C. Jyothilekshmi S. S. V. College, Valayanchirangara, Ernakulam, Kerala, India

Nandakumar Kalarikkal Department of Pharmaceutics, Mahatma Gandhi University, Kottayam, India

School of Pure and Applied Physics, Mahatma Gandhi University, Athirampuzha, Kerala, India

Hanieh Kargarzadeh Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Lodz, Poland

Varsha Khare Soft Foundry Institute, Seoul National University, School of Engineering, Seoul, South Korea

Hyun-Joong Kim Smart Center for Adhesion Research & Development (Smart CARD), Laboratory of Adhesion & Bio-Composites, Program in Environmental Materials Science, College of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea

Amee Krishnakumar Institute of Science, Nirma University, Ahmedabad, Gujarat, India

Veda Krishnan Division of Biochemistry, ICAR – Indian Agricultural Research Institute (IARI), New Delhi, India

Devu P. Kumar Centre for Innovations and Technologies (CIT), ADSO Naturals Private Limited, Bangalore, India

Terrence Madhujith Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka

Akshit Malhotra Department of Microbiology, Tripura University (A Central University), Agartala, Tripura, India
Invisiobiome, New Delhi, India

Asanga Manamperi Materials Engineering Department, California Polytechnic State University, San Luis Obispo, CA, USA

Department of Chemical Engineering, College of Engineering, Kettering University, Flint, MI, USA

Sudhagar Mani School of Chemical, Materials and Biomedical Engineering, University of Georgia, Athens, GA, USA

B. Brailson Mansingh Sri Ramakrishna Engineering College, Coimbatore, India

Alson Mart Post Graduate and Research Department of Chemistry, Maharaja's College, Ernakulam, India

Krishnakumar Melethil Department of Chemistry, Newman College, Thodupuzha, Kerala, India

Liliana Melro Centre for Textile Science and Technology (2C2T), University of Minho, Guimarães, Portugal

Anju Mohan S. S. V. College, Valayanchirangara, Ernakulam, Kerala, India

Debarati Mondal Division of Biochemistry, ICAR – Indian Agricultural Research Institute (IARI), New Delhi, India

Nazish Mumtaz School of Chemistry, University of the Punjab, Lahore, Pakistan

Suja N. R. Post Graduate and Research Department of Chemistry, Maharaja's College, Ernakulam, India

Ashok Kumar Nadda Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Wagnaghat, Solan, Himachal Pradesh, India

D. H. Nagaraju Department of Chemistry, School of Applied Sciences, Reva University, Bangalore, India

Amrutha S. Nair Centre for Innovations and Technologies (CIT), ADSO Naturals Private Limited, Bangalore, India

Ajalesh B. Nair Department of Chemistry, Union Christian College, Aluva, India
Department of Chemistry, Sri Vyasa N. S. S. College, Wadakkanchery, India

Nisha Nandakumar Department of Chemistry, Union Christian College, Aluva, India

Department of Chemistry, Sri Vyasa N. S. S. College, Wadakkanchery, India

Deepa Narayanan Cochin University of Science and Technology (CUSAT), Cochin, India

M. Nishaf Naseeha Farsana Post-Graduate and Research Department of Chemistry, Maharajas College, Ernakulam, India

Henry Ndibe Department of Chemistry, University of Benin, Benin City, Nigeria

P. S. Neetha CUSAT, Cochin, India

P. Nisha Department of Biosciences, MES College, Marampally, Ernakulam, Kerala, India

Brendan C. O'Kelly Department of Civil, Structural and Environmental Engineering, Trinity College Dublin, Dublin, Ireland

Jyothi P. R. Department of Chemistry, M A College, Ernakulam, Kerala, India

Rejimon P. K. Post Graduate and Research Department of Chemistry, Maharaja's College, Ernakulam, India

Vinod V. T. Padil Amrita School for Sustainable Development (AST) / Amrita Vishwa Vidyapeetham, Amrita University, Kollam, Kerala, India

Jorge Padrão Centre for Textile Science and Technology (2C2T), University of Minho, Guimarães, Portugal

Puneet Pathak Avantha Centre for Industrial Research and Development (ACIRD), Thapar Institute of Engineering and Technology Campus, Patiala, India

Anju Paul Department of Chemistry, Sree Sankara Vidyapeetom College, Perumbavoor, India

Deepalekshmi Ponnammam Materials Science and Technology Program, Department of Mathematics, Statistics and Physics, College of Arts and Sciences, Qatar University, Doha, Qatar

Elisabeta Elena Popa Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine Bucharest, Bucharest, Romania

R. Rajakumari Department of Pharmaceutics, Krupanidhi College of Pharmacy, Bengaluru, Karnataka, India

Deenadhayalan Rajalakshmi Department of Biotechnology, School of Bio and Chemical Engineering Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

Sanjay Preeth Ram Singh Department of Biotechnology, School of Bio and Chemical Engineering Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

Arooj Ramzan School of Chemistry, University of the Punjab, Lahore, Pakistan

Maria Râpă Faculty of Materials Science and Engineering, University POLITEHNICA of Bucharest, Bucharest, Romania

T. A. Raslana Department of Biosciences, MES College, Marampally, Ernakulam, Kerala, India

K. A. Razeena Department of Biosciences, MES College, Marampally, Ernakulam, Kerala, India

Ana Isabel Ribeiro Centre for Textile Science and Technology (2C2T), University of Minho, Guimarães, Portugal

Noor Afizah Rosli Department of Chemical Science, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia

Roshny Roy Department of Chemistry, Newman College, Thodupuzha, India

M. H. Rubiya Mahatma Gandhi University Centre for Research in Chemistry, Mar Athanasius College, Kothamangalam, Kerala, India

Kwang-Hyun Ryu Smart Center for Adhesion Research & Development (Smart CARD), Laboratory of Adhesion & Bio-Composites, Program in Environmental Materials Science, College of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea

Hema S. Department of Chemistry, Amrita Vishwa Vidyapeetham, Amritapuri, Kollam, Kerala, India

Amrutha S. R. Post Graduate and Research Department of Chemistry, Maharaja's College, Ernakulam, India

Malavika Sajith Department of Chemistry, Amrita Vishwa Vidyapeetham, Amritapuri, Kollam, Kerala, India

Sreedha Sambhudevan Department of Chemistry, Amrita Vishwa Vidyapeetham, Amritapuri, Kollam, Kerala, India

Antony V. Samrot School of Bioscience, Faculty of Medicine, Bioscience and Nursing, MAHSA University, Jenjarom, Selangor, Malaysia

P. K. Sandhya School of Chemical Sciences, Mahatma Gandhi University, Kottayam, India

P. V. Sandhya Post-Graduate and Research Department of Chemistry, Maharajas College, Ernakulam, India

Sreesha Sasi Department of Chemistry, Maharaja's College, Ernakulam, India

Mahendran Sathiyasree Department of Biotechnology, School of Bio and Chemical Engineering Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

S. Shabanamol SAFI Institute of Advanced Study, Malappuram, Kerala, India

Kavya Shah Institute of Science, Nirma University, Ahmedabad, Gujarat, India

Chhavi Sharma Avantha Centre for Industrial Research and Development (ACIRD), Thapar Institute of Engineering and Technology Campus, Patiala, India

Sreekala S. Sharma Department of Chemistry, Sree Sankara Vidyapeetom College, Perumbavoor, India

Swati Sharma University Institute of Biotechnology, Chandigarh University, Mohali, Punjab, India

P. S. Shasiya Department of Chemistry, Union Christian College, Aluva, Kerala, India

Urja Shedaliya Institute of Science, Nirma University, Ahmedabad, Gujarat, India

Nagarajan Shobana Department of Biotechnology, School of Bio and Chemical Engineering Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

K. Simi Pushpan Department of Chemistry, Union Christian College, Aluva, Kerala, India

J. Sindhu Rachel Department of Botany, Nirmala College, Muvattupuzha, India

Archana Singh Division of Biochemistry, ICAR – Indian Agricultural Research Institute (IARI), New Delhi, India

Ruksana Sirach Department of Chemistry, Sardar Patel University, Gujarat, India

Shree Krithika Sivasuriyan Department of Biotechnology, School of Bio and Chemical Engineering Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

Xinyu Song Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Qingdao, China

Sanjiv Sonkaria Laboratory of Adhesion & Bio-Composites, Program in Environmental Materials Science, Seoul National University, Seoul, Republic of Korea
Soft Foundry Institute, Seoul National University, School of Engineering, Seoul, South Korea

K. Sreedevi Department of Chemistry, Sree Sankara Vidyapeetom College, Valayanchirangara, Kerala, India

M. S. Sreekala School of Chemical Sciences, Mahatma Gandhi University, Kottayam, India

B. S. Subi Indira Gandhi College of Arts and Science, Kothamangalam, Kerala, India

Evangelin R. Sujatha Centre for Advanced Research in Environment, School of Civil Engineering, SASTRA Deemed to be University, Thanjavur, India

Heru Suryanto Center of Excellent for Cellulose Composite (CECCom), Faculty of Engineering, Universitas Negeri Malang, Malang, Indonesia

M. Thangavel School of Life Sciences, Nehru College of Arts & Sciences, Coimbatore, Tamilnadu, India

Punniamoorthy Thiviya Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka

Bejoy Thomas Department of Chemistry, Newman College, Thodupuzha, Kerala, India

Sabu Thomas School of Chemical Sciences, Mahatma Gandhi University, Kottayam, Kerala, India

School of Energy Materials, Mahatma Gandhi University, Kottayam, Kerala, India

Department of Pharmaceutics, Mahatma Gandhi University, Kottayam, India

Ritika Tripathi Department of Applied Science, School of Engineering and Technology, BML Munjal University, Gurugram, Haryana, India

Greeshma U. Chandran Department of Chemistry, Amrita Vishwa Vidyapeetham, Amritapuri, Kollam, Kerala, India

Ragitha V. M. KSCSTE-Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Thiruvananthapuram, India

Rini Thresia Varghese School of Energy Materials, Mahatma Gandhi University, Kottayam, India

Sharon Varghese Department of Chemistry, Newman College, Thodupuzha, Kerala, India

B. Venugopal Post Graduate and Research Department of Chemistry, Maharaja's College, Ernakulam, Kerala, India

Sneha Xavier Department of Biotechnology, School of Bio and Chemical Engineering Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

Uun Yanuhar Department of Aquatic Resources Management, Brawijaya University, Malang, Indonesia

Swathi Yempally Center for Advanced Materials, Qatar University, Doha, Qatar

Zhoveta Yhobu Centre for Nano and Material Sciences, Jain University, Bangalore, India

Liangmin Yu Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Qingdao, China

Andrea Zille Centre for Textile Science and Technology (2C2T), University of Minho, Guimarães, Portugal

Part I

Introduction



Biopolymers

1

State of the Art and New Challenges

Bejoy Thomas and Krishnakumar Melethil

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Abstract

Biopolymers, as opposed to petroleum, the conventional source of polymers, are those that are created by or obtained from living creatures, such as plants and bacteria. Given their applications to numerous facets of human existence, biopolymers, one of the most diverse groups of organic compounds, have recently attracted a lot of study interest. Although only partially, these molecules and the materials created with them have replaced the chemical polymers and materials sourced from petroleum. For instance, nanocellulose, which is regarded as the material of the twenty-first century, has applications in a variety of fields (or facets) of human life, including advanced biomedical, packaging, gas-sensing, and electronic ones. Today, biopolymers are among the study fields that have been most thoroughly explored due to the increasing interest in this sector from academic and industrial researchers.

B. Thomas (✉) · K. Melethil

Department of Chemistry, Newman College, Thodupuzha, Kerala, India

e-mail: bejoy.thomas@newmancollege.ac.in

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Bacterial biopolymers

Introduction

The field of polymer science, often known as polymer chemistry, is extremely prevalent and important in the modern world. To distinguish it from other human eras that were dominated by a variety of different materials, it has even been suggested to refer to the current period as the “polymer age” (the Stone, the Bronze, and the Iron Ages, classical era, etc.). The advent and widespread adoption of polymer technology have gradually affected current world perspectives and discourses, much like earlier revolutionary technical developments like the advancement of the computer and more modern humanoids powered by artificial intelligence (Brydson 1999; Campbell 2002). Despite the astounding advancements in polymer science that have been made over the years, including those in simple household applications, computers, AI, and MI, there are still many fascinating challenges that need to be solved. These challenges are worthwhile to solve due to their effects on our daily lives and, more specifically, the environment. Traditional examples of problems include issues with environmental contamination and a lack of efficient disposal methods, micro- to nanoscale plastics and their intended design for long life spans, polymers for energy depository and delivery, polymers for the human body, green polymer chemistry and technology, etc. (Limaye et al. 2011; Cella et al. 2022).

When plastic (especially the nonreusable form) is discharged into the environment after use, several of the polymers’ strikingly favourable characteristics, such as their chemical, physical, and biological inertness and durability or life span under environmental conditions, offer problems. Common plastics like polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), thermoplastic polyurethanes (TPU), polypropylene (PP), polytetrafluoroethylene (Teflon), and polyethylene terephthalate (PET) are exceedingly tenacious in the environment, slowly disintegrating into tiny particles over decades by optical, physical, and biological degradation processes. A stage of the degradation process that cannot be avoided is the fragmentation of the substance into smaller and smaller particles. In 2020, Min et al., developed a data-driven method for illuminating plastic debris degradation trends by tying physical characteristics and molecular structures to biotic and abiotic degradation behavior in saltwater. The findings show a hierarchy of predictors to measure surface erosion as well as combinations of characteristics, such glass transition temperature and hydrophobicity, to divide ocean plastics into fast, medium, and slow degradation groups.

As a result, both developing and developed nations now face a threat from the requirement for effective waste disposal due to the growing use of polymeric materials and plastics made from fossil fuels. There are serious environmental

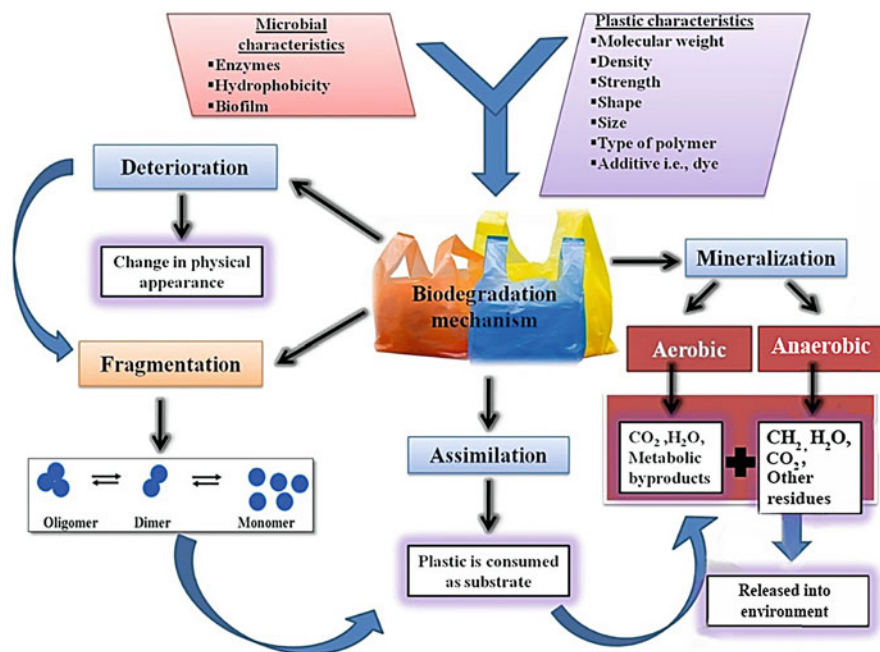


Fig. 1 Schematic representation of the consolidated mechanism of microbial degradation of plastics under environmental conditions such as soil-bound microorganisms into degradation products including CO_2 , CH_4 , etc., some potent greenhouse gases

concerns as a result of the over usage of plastic and the inappropriate methods of disposal used in many nations. The systems and procedures used for this process of disposing of plastic through various chemical and physical processes are very expensive and produce organic pollutants, such as micro- or nanoplastic degradation products, which worsen the environment (Lambert et al. (2014); Azapagic et al. (2003)). Using the biodegradation of common plastics like polyethylene terephthalate (PET), polyethylene (PE), and polystyrene (PS) under various environmental conditions as an example, recent studies have demonstrated that using enzymes and various microbes for assimilation and disintegration, respectively, is a viable bioremediation pathway (Fig. 1).

The field of polymer science, often known as polymer chemistry, is extremely prevalent and important in the modern world. To distinguish it from other human eras that were dominated by a variety of different materials, it has even been suggested to refer to the current period as the “polymer age” (the Stone, the Bronze, and the Iron Ages). Without polymers, modern lifestyle is rarely imaginable. Just like with other major technological advancements, such as the creation of the computer and more contemporary humanoids based on artificial intelligence, the advent and widespread establishments of polymer technology have dramatically altered the present world’s perceptions and discourses over time (Brydson 1999;

Campbell 2002). Despite the astonishing achievements of polymer science witnessed over the years, from simple household applications to computers to AI to MI, many exciting challenges remain to be addressed; these are well worth to tackle because of their impact on our everyday life and, more accurately, the ecology. Classical examples of challenges include environmental pollution issues and a lack of effective disposal mechanism, micro- to nanoplastics and designed designation of long life times, polymers for energy storage and delivery, polymers for the human health, green polymer chemistry, etc. (Cella et al. 2022; Singh et al. 2020).

Unfortunately, many of the plastics' strikingly positive qualities – such as their durability, longevity, and chemical, physical, and biological inertness – present problems when plastic, especially the nonreusable kind, is discharged into the environment after use. The degradation of typical plastics like polypropylene (PP), polyethylene (PE), polystyrene (PS), polyvinyl chloride (PVC), thermoplastic polyurethanes (TPU), polypropylene (PP), polytetrafluoroethylene (Teflon), and polyethylene terephthalate (PET) by optical, physical, and biological processes causes a very slow fragmentation of the material into minute particles, which is thought to take millennia. A stage of the degradation process that cannot be avoided is the fragmentation of the substance into smaller and smaller particles.

Therefore, the requirement for effective waste disposal has become a threat to both developing and developed countries due to the growing usage of polymeric materials and plastics made from fossil fuels worldwide. In many nations across the world, excessive plastic consumption and a poor method of disposing it have raised serious environmental issues. Disposal system and methods adopted for this process of plastic through various chemical and physical processes are very expensive which also produce organic pollutants including micro- or nanoplastic degradation products, resulting in environmental deterioration (Azapagic et al. 2003; Lambert et al. 2014). For instance, recent studies on the biodegradation of common plastics like polyethylene terephthalate (PET), polyethylene (PE), and polystyrene (PS) in various environmental settings have shown that using enzymes and different microbes for assimilation and disintegration, respectively, is a practical bioremediation pathway (Fig. 1).

Biodegradable Polymers

The creation and manufacture of so-called biodegradable plastics, which could have shorter residence duration under environmental conditions, have received a lot of attention during the past couple of decades. One type of polymers that is frequently pushed under the banner of “biodegradable” plastics is “oxo-degradable” plastics. In actuality, these are common plastics (including PE, PS, PP, and PET) that contain prodegradants or additives that may speed up the oxidation process. Oxo-degradable plastics offer a significant problem because, when exposed to sunshine and oxygen,

they quickly fragment into massive amounts of microplastics. While it helps large plastic materials degrade more quickly than conventional plastics in 2010 (O'Brine and Thompson 2010), the produced microplastic is identical to any other type of microplastic from conventional plastics and takes a long time to fully biodegrade, continuing to pose a threat to the environment (Tosin et al. 2012).

Internationally accepted standard procedures that enable biodegradability to be assessed over a range of typical environmental matrices are available to aid in the creation of biodegradable plastics (including marine, soils, wastewater treatment plants, etc.). The biodegradability of plastics (biodegradable) under ideal industrial and municipal composting circumstances can also be evaluated using standard requirements and test techniques (e.g., ASTM D6400, EN 13432, ASTM D5338, ASTM D5929). Plastics that fully mineralize over a reasonable period of time in industrial composting facilities' high temperatures and controlled conditions can be regarded as biodegradable or compostable. The process might be profitable because these components of the compost are also valued commercially (Fig. 2). Additionally, because the degradation occurs inside of a closed and controlled system, the fragmentation occurs quickly in an industrial composter and poses little risk for microplastics to be exposed to the environment (Chamas et al. 2020). As of right now, there are only a very limited number of products made of compostable polymers, such as bags, take-out containers, coffee pods, food packaging, cups, plates, and service ware, as well as items made of biodegradable polymers like polyglycolic acid (PGA), polycaprolactone (PCL), poly(vinyl alcohol) (PVA, PVOH), polylactic acid (PLA), and polybutylene succinate (PBS). The proportion of biodegradable and compostable plastics that are composted as opposed to ending up in common waste streams, however, does not appear to be well-documented globally (Andersen 2007). In fact, the volume of composted material must be much less than the vast amount of (bio)degradable polymers generated globally (ca. 367 million metric tonnes in 2020).



Fig. 2 An on-the-field image of a composting facility in the United States for compostable products including food packaging materials and biodegradable polymers including polyglycolic acid (PGA), etc.

Biopolymers

Biopolymers, as opposed to synthetic polymers formed from petroleum and biodegradable polymers, can be produced using materials that are perpetually renewable, sustainable, and carbon-neutral. Because the majority of these components are derived from agricultural crops, their utilization might develop a sustainable sector. Biopolymers are organic materials made by living things' cells. These molecules are made up of monomeric components that join or together through covalent bonds to form bigger molecules. Depending on the type of monomeric molecule that contributes to the entire structure of the polymer, three *categories can be found, namely*, polynucleotides, polypeptides, and polysaccharides, which are further divided based on the monomers employed and the final biopolymer structure. DNA and RNA are 2 examples of polynucleotides, which are lengthy polymers made up of 13 or more nucleotide monomers. Proteins and polypeptides are polymers of amino acids, with collagen, actin, and fibrin serving as some prominent examples. Examples of polysaccharides, which are linear or branched polymeric carbohydrates, include starch, cellulose, heparin, and alginate, among others. Other types of biopolymers include natural rubber (isoprene polymers); chitin, which is the second most prevalent natural polysaccharide after cellulose and is a linear polymer made up of (1,4)-N-acetylglucosamine units; suberin and lignin, which are complex polyphenolic polymers; cutin and cutan, which are complex polymers of long-chain fatty acids; and melanin, which produces hair, eye, and skin pigmentation (Niaounakis 2015; Baranwal et al. 2022). Figure 3 illustrates a broad taxonomy of biopolymers.

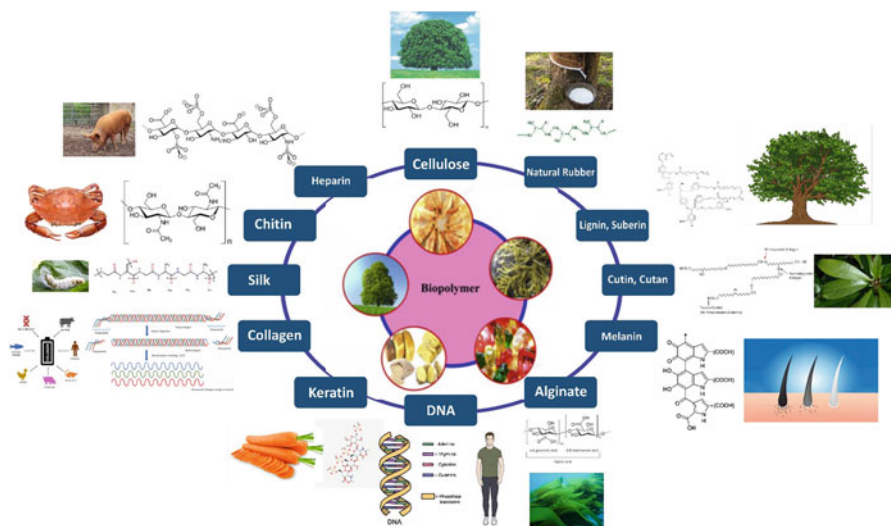


Fig. 3 Schematic representation of the general broad classification of biopolymers based on the types and the potential sources

Such naturally derived molecules/materials from strongly hierarchical organization are appealing candidates for high-performance and functional advanced materials because of their renewable nature, nontoxicity, biocompatibility, biodegradability, flexibility, greater availability, cost-effectiveness, and eco-friendliness as compared to non-biodegradable petroleum-based polymers (Niaounakis 2015). Similar bio-enabled nanocomposites constructed of biopolymers also have these intrinsic properties, but the synergistic combination of biological and synthetic components made feasible by strong interfacial contacts can greatly enhance structural performance and enable new activities. Therefore, compared to synthetic polymers, these natural materials have multiple advantages, specifically in the social, economic, and environmental domains. Many biopolymers have been compared with petroleum-based (bio)polymers using life cycle assessment (LCA; life cycle assessment is a cradle-to-grave or cradle-to-cradle analysis technique to assess environmental impacts associated with all the stages of a product's life, which is from raw material extraction through materials processing, manufacture, distribution, and use). The results are in line with expectations and demonstrate that biopolymers have specific, well-defined environmental benefits (Yates and Barlow 2013). Additionally, compared to biopolymers, petroleum-based polymeric goods have a far greater negative influence on the sustainability of the environment. Advanced uses of biopolymers in (bio)medicine, biomedical engineering, food coatings, food packaging, tissue engineering, manufacturing, military, and environmental applications imply that they are better for the environment than synthetic polymers (Baranwal et al. 2022).

Carbohydrate-Based Biopolymers (Marine and Terrestrial)

Biopolymers, particularly those based on carbohydrates and proteins, had already seen extensive use in the food and pharmaceutical industries much prior to the emergence of the petroleum industry. Biomacromolecules called polysaccharide biopolymers, which can thicken, cross-link, adsorb, and perform other diverse functions, are obtained from renewable resources. These molecules are made up of O-glycosidic links that connect monomeric units of sugars or their simple derivatives (such as glucose and its derivatives found in cellulose and chitin, respectively) to build bigger structures. The characteristics and possible applications of this class of biopolymers are determined by the characteristics of the monomers, the types of connections, and any potential chemical alterations of the surface (Verma et al. 2020). Some of the crucial members of this class are cellulose, starch, epoxy-polysaccharides, and marine chitin.

Marine prokaryotes, including bacteria and archaea, present a wide range of opportunities for the creation of new biopolymers. Numerous innovative material characteristics and business prospects are provided by these biopolymers. Hyaluronic acid (a well-known heteropolysaccharide or heteroglycan), chitin and chitosan, peptides, collagen, enzymes, polysaccharides from algae, and secondary metabolites like mycosporines are among the marine macromolecules. The

distinctive qualities of marine products, such as exopolysaccharides and melanins, which improve the ability of the organisms producing them to survive, can be utilized for a variety of products, from emulsifiers to adhesives. Polyhydroxyalkanoates are the molecules that prokaryotes use to store carbon, but their technical use is altogether different because it might be used as a base for biodegradable plastics. Epoxypolysaccharides from microorganisms (EPS) are a class of significant goods that are gaining interest from a wide range of industrial sectors. Although several known marine bacteria create exopolysaccharides, the ongoing hunt for microorganisms that produce polysaccharides shows promise. According to the screening of a variety of mesophilic heterotrophic bacteria obtained from various places, hydrothermal deep-sea vents might be a source of new EPS. Some bacteria have been demonstrated to biosynthesize novel EPS in a lab setting while coming from such a harsh environment. Deep-sea *Alteromonas infernus*, deep-sea *Alteromonas macleodii* strain, *Alteromonas* strains obtained from French Polynesia marine environments, etc. all generate epoxypolysaccharide. Marine EPS-producing bacteria have also been isolated from a number of extreme niches, including the frigid marine habitats typical of Arctic and Antarctic sea ice, which are characterized by low temperature and low nutrient content. Finally, it may be said that marine biopolymers represent a large and underutilized biological resource (Grandics 1981; Kaur and Singh 2020).

The manufacture of chitosan often requires an alkali or enzymatic treatment to deacetylate chitin, which is the second most frequent structural polysaccharide found in nature after cellulose. It is the most prevalent renewable polymer in the oceans and provides vital carbon and nitrogen for marine life. The cycling of nutrients in the oceans involves the process of chitin breakdown, and chitinolytic bacteria are crucial players in this cycle. Chitin's natural form is that of organized crystalline microfibrils, which serve as structural elements, primarily in the exoskeleton of arthropods (Fig. 4). Since shrimp and crabs and other crustaceans produce chitin, chitin is separated from their exoskeletons. It has been identified that the β -form of chitin, which is found in squid and has been shown to be more susceptible to deacetylation. Alternative sources of chitin/chitosan can also include some species of bacteria, fungi, algae, and insects. With annual production exceeding 1011 tonnes, chitin is regarded as the primary biomass resource. Chitin can be used as a biosurfactant because it has both hydrophilic and lipophilic components, making it an amphiphilic molecule. It can self-assemble into nanometric-sized fibers. Chiral nematic self-assembly of chitin nanofibers has been reported, and it uses the so-called natural route, which forgoes chemical etching in favour of calcium ions (Ravi Kumar 2000; Narkevicius et al. 2019).

Cellulose is a highly sustainable raw material for a variety of chemical applications due to its abundance as a renewable natural resource and its intriguing structure, characteristics, and activities. Due to its inherent qualities, renewability, ease of functionalization, and diversity brought on by natural abundance, nanocellulose (NC) has emerged as one of the most promising green materials of the modern era. Choosing lignocellulosic biomass, an agricultural waste, as the cellulose source makes it more appropriate. Producing cellulose and NC from biomass

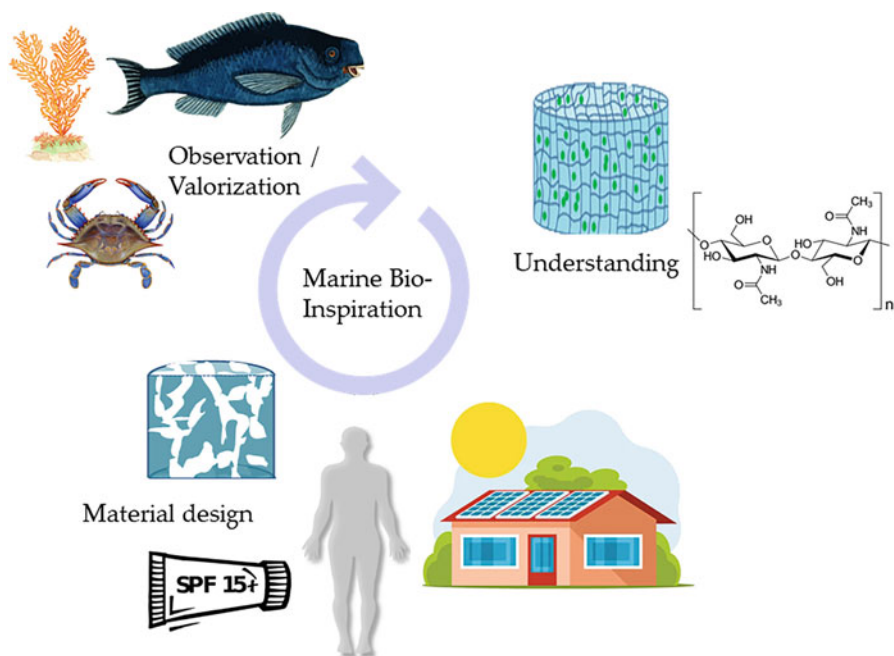


Fig. 4 Schematic representation of the marine bioinspiration, which potentially produce marine biopolymers, including chitin

feedstocks, microbial cultures, and plant materials like cotton, softwood, bark, and leaves can be done in a variety of chemical, chemo-mechanical, biochemical, and combination processes. Due to many of cellulose's well-known desirable properties, such as low density, nontoxicity, biodegradability, flexibility, affordability, environmental friendliness, the availability of multiple reactive sites for introducing novel functionalities, thermal stability, mechanical properties, reinforcing abilities, and tunable self-assembly in aqueous media, NC have attracted considerable interest. It is possible to divide the resulting nanocellulose into cellulose nanocrystals (CNCs), cellulose nanofibers (CNFs), and bacterial nanocellulose (BNC). The source and extraction technique used during the production stages affect the physical qualities of nanocellulose. Strong mineral acids are used to hydrolyze cellulose to produce cellulose nanocrystals; cellulose nanofibers are separated mechanically or chemically; and bacterial cellulose is produced biochemically. A crucial step in implementing sustainability into the extraction process is substituting the destructive sulfuric acid used during acid hydrolysis, and alternative technologies utilized at this point have been thoroughly investigated. Its numerous applications are made possible by characteristics including its high surface area and aspect ratio, rheological behaviour, capacity to absorb water, crystallinity, customizable surface chemistry, and, most importantly, its lack of cytotoxicity and genotoxicity. One of the main benefits of nanocellulose is its highly functionalized surface. Functional groups'

(primarily the $-CH_2$ group) decoration by various methods like esterification, etherification, amidation, urethanization (hydrophobic decorations), phosphorylation, sulfonation, TEMPO-oxidation, and carboxymethylation (hydrophilic surface modifications) have frequently been done over nanocellulose for various cutting-edge applications, such as biomedical. Tissue engineering, antimicrobial coating, wound dressings, medication delivery, and other innovative uses of NC-based materials in the field of medicine are just a few (Thomas et al. 2018).

Nanocellulose can be derived from a variety of sources, including plants, microbes, algae, and aquatic animals (tunicates). A comparison of the four different types of nanocellulose types is shown in Fig. 5. When compared to other extraction methods, *Cladophora* nanocellulose has distinct characteristics like high crystallinity ($>95\%$), large fiber aspect ratio, large surface area, and good mechanical and rheological properties (Zhou et al. 2019). It is very important to use dried, redispersible cellulose nanomaterials for many specific applications, and in this sense *Cladophora* nanocellulose is known to have a higher level of redispersibility. Green algae called *Cladophora* sp. have the ability to be synthesized and extracted as

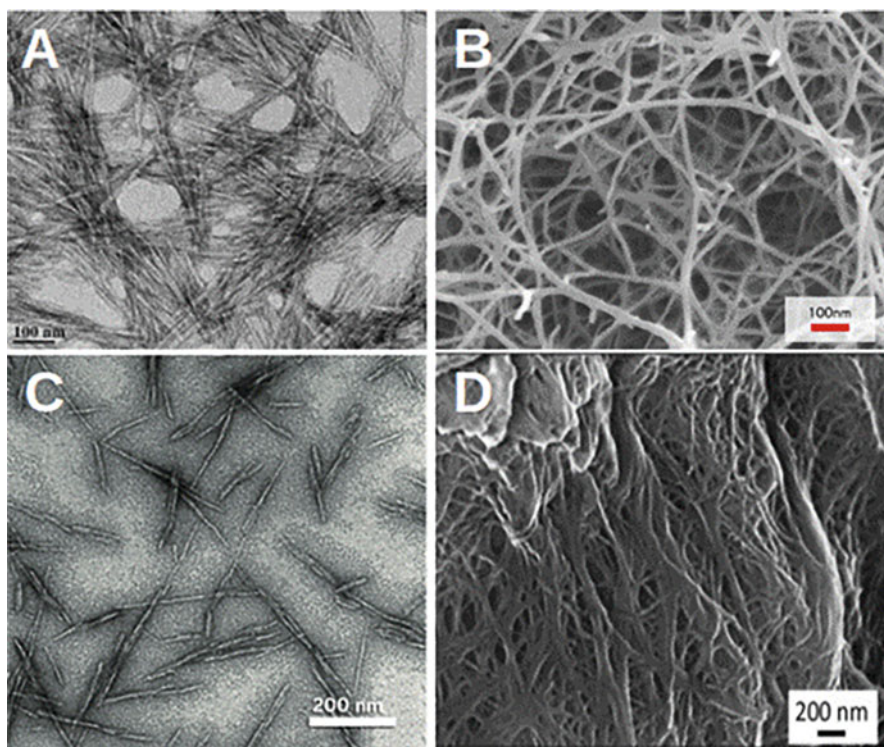


Fig. 5 TEM images demonstrating the morphological features of four different types of nanocelluloses: (a) cellulose nanocrystals (CNC), (b) cellulose nanofibers, (c) bacterial nanocellulose, and (d) *Cladophora* nanocellulose

nanometer-sized crystalline cellulose (Sucaldito and Camacho 2017). *Cladophora* cellulose (CC) is simple to extract and offers exceptional solution processability, low moisture adsorption capacity, adjustable mesoporosity, high surface area, high chemical stability, low moisture absorption, robust and flexible mechanical capabilities, and diverse surface modifiability. These distinctive physical and chemical characteristics of CC can be used to create new features and improve the performance of nanocellulose-based materials, providing advantages over other sources in application areas like energy, environmental science (such as water and air purification, pollutant adsorption), and the life sciences (virus isolation, protein recovery, hemodialysis, DNA extraction, bioactive substrates, etc.). Over the past ten years, these characteristics have garnered a lot of study attention, particularly in the fields of energy storage (sustainable paper-based energy storage devices), environmental science, and life science. For instance, using CC-based separators during device manufacturing can improve the safety, stability, and capacity of lithium-ion batteries (Suciyati et al. 2022).

Heteropolysaccharide-Based Biopolymers (Animal and Plant)

Heteroglycans are heteropolysaccharides that typically contain two or more distinct monosaccharide units; however, some of the more uncommon examples may have three or more. A majority of naturally occurring heteroglycans, however, only have two distinct monosaccharide units and are intimately linked to lipid or protein. The tremendous complexity of the nature of these kinds of polysaccharides has produced the ideal environment for thorough structural research. Such research are, however, very challenging. The main heteropolysaccharides are the blood group substances (blood group antigens, which can be either proteins (Rh, Kell) or glycoproteins and glycolipids (ABO)), the connective-tissue polysaccharides (also known as glycosamino-glycans), and the connective-tissue heteropolysaccharides (contain acidic groups (uronic acids or sulfate groups)) and can bind water. Glycoproteins (combinations of carbohydrates and proteins) like gamma globulin; glycolipids (combinations of carbohydrates and lipids), especially those found in the central nervous system of animals; and a variety of plant gums (Fig. 6) can all play a part in other physiological processes, such as the accumulation of calcium prior to bone formation (Glasser 2008).

The most significant types of heteropolysaccharides can be found in the connective tissues of all mammals. These huge molecules that are included in this class of heteropolysaccharides range in size, shape, and how they interact with other components of the body. Individual connective-tissue polysaccharides have structural roles, and these roles are tied to particular animal functions. For example, hyaluronic acid, the main component of joint fluid in animals, serves as a lubricant and a shock absorber. These molecules can easily bind both water and inorganic metal ions because they have acidic groups (such as uronic acids or sulfate groups). They may also be involved in other physiological processes, such as the build-up of calcium prior to bone formation. Heparin, a heterogenic mixture of sulfonated

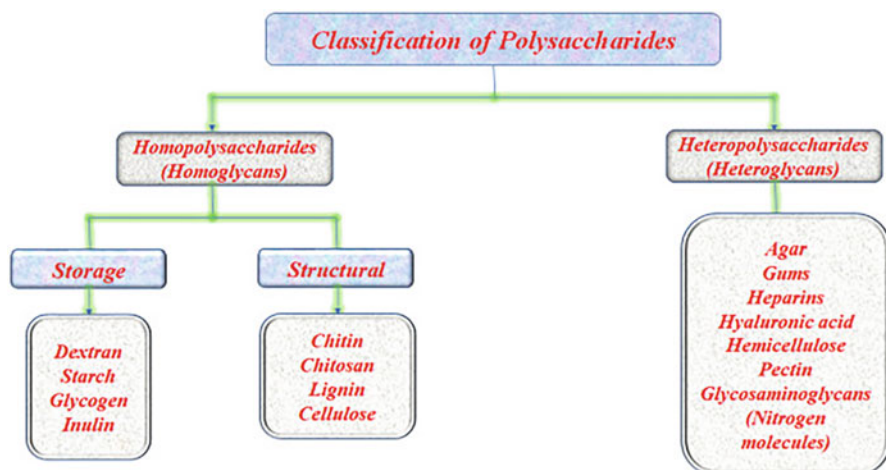


Fig. 6 An illustration showing the classification of polysaccharides into homo- and heteropolysaccharides with corresponding examples for each class

polysaccharides made from repeating units of D-glucosamine, D-glucuronic acid, and L-iduronic acid, is used to prevent or treat a variety of blood vessel, heart, and lung conditions. Ion-binding capacity also seems to be connected to heparin's anticoagulant activity. Additionally, extensive surgical procedures including open-heart surgery, bypass surgery, and kidney dialysis and blood transfusions all involve the use of heparin to stop blood clotting (Gan et al. 2017).

Agar is made up of dry, sulfuric acid esters that are water-soluble and is extracted from several *Gelidium* species (red algae). Agar's gel strength is derived from two different types of heterogeneous polysaccharides: (1) agarose constitutes up about 70% of the mixture and is a linear polymer made up of repeating units of agarobiose, a disaccharide made up of D-galactose and 3,6-anhydro-L-galactopyranose (it forms the supporting structure in the cell walls of certain species of algae), and (2) agaropectin, a sulfonated polysaccharide in which galactose and uronic acid units are not entirely esterified with sulfuric acid. Agar swells when treated with cold water, dissolves in boiling water, and forms gel on cooling. Some of the most widespread uses include making bacterial culture media, acting as an emulsifier, treating constipation, acting as a bulk laxative, and thickening ice cream, among others. It is also used in the preparation of vaginal capsules and suppositories, which is a cone-shaped capsule-like structure with the medicine in it, to be introduced into the rectum, urethra, or vagina (Williams and Phillips 2000; Cui 2001).

Galacturonic acid and arabinose make up pectin, which has a very complicated molecular weight. It is created during the ripening of citrus fruits and is typically isolated from the pulp of fruits like lemons and oranges, whose structures noticeably shift during ripening. Its ability to conjugate toxins and absorb them makes it a protective substance with some of the most important applications in the treatment of intestinal illnesses like diarrhea and dysentery. It is employed in pharmacies as a gel

and emulsion stabilizer as well as the creation of jams and jellies (Izydorczyk et al. 2005). Naturally occurring polysaccharides, such as those found in natural gums, have the ability to significantly improve a solution's viscosity even at low concentrations. As thickening agents, gelling agents, emulsifying agents, and stabilizers, these are employed in the food sector. As adhesives and binding agents, they are widely utilized in various sectors. These compounds are created in higher plants either spontaneously or as a result of mechanical harm as a kind of post-injury protection (pathological). Gum tragacanth and gum arabic are two of the most prevalent examples (G. tragacanth). The characteristic of gums to swell in water and transform into viscous, sticky, or adhesive solutions is a common one. These are neutral salts of complicated polysaccharide acids that have residual uronic and sugar acids. Pentose (D-xylose, L-arabinose), methylpentose (e.g., L-rhamnose), hexose (D-mannose, D-galactose), and uronic acid residues are some frequent ingredients, such as D-glucuronic or galacturonic acid (Cui 2001).

The majority of research on mushrooms has focused on their heteropolysaccharides, which are a class of more complicated polymers in addition to their D-glucans. It's possible to view mushroom heteropolysaccharides as a fascinating source of compounds with both industrial and medical uses. The types of polysaccharides that are obtained from various sources of mushroom-forming fungus, such as Basidiomycetes, Ascomycetes, hybrid mushrooms, etc., exhibit a tremendous diversity. The many polymers discovered in these mushrooms are structurally analyzed to determine the heteropolysaccharides. These polymers can be divided into heterogalactans, heteroglucans, and heteromannans depending on the primary chain they contain (Ruthes et al. 2016).

The backbone of polymers typically consists of (1–6)-linked D-galactopyranosyl residues, some of which have side chains made of L-fucopyranosyl, D-mannopyranosyl, D-galactopyranosyl, or 3-O- α -D-mannopyranosyl- α -L-fucopyranosyl residues. When D-Manp as end units are absent, the various sorts of substitutions give heterogalactans, such as fucogalactans, different names. The main chain of heterogalactans can also be partially O-methylated, displaying 3-O-Me-D-Galp units. Natural sugars may be etherified by various groups, but methyl ethers are the most frequently used. The most common polysaccharides found in mushrooms are D-glucans, specifically in a particular configuration. But other scientists hypothesized the existence of heteroglucans, which had branches made up of several monosaccharides and a core chain made of D-glucopyranose residues. The structures described thus far typically consist of -D-Glcp residues that are (16)-, (14)-, and/or (13)-linked, with terminal non-reducing residues of β -d-Glcp; α -D-Galp; α -L-Fucp; and α -D-Glcp replacing them at O-3 or O-6. In polymers derived from various sources, disaccharides or tiny branches of monosaccharide assemblies were also seen. *Lepista sordida* produced α -D-glucan, substituted at O-2 by α -D-Galp residues substituted at the O-2 position, which was described as having the most complicated structure of all the mushroom heteroglucans hitherto reported (Shirakawa et al. 1998; Luo et al. 2012). Common polysaccharides like mannans and heteromannans are found primarily in Ascomycetes. They make up a portion of the fungus' cell wall structure and can be joined to proteins to create the cell wall matrix (Netea

et al. 2008). Galactomannans, glucogalactomannans, and xylomannans are three examples of the heteromannans that have been isolated from mushrooms. Extreme complexity was revealed by structural analyses on a variety of heteromannans that were isolated from diverse mushrooms. There aren't many studies on heteromannans, and their complicated characteristics made purifying them difficult.

Peptide- or Protein-Based Biopolymers

For various reasons, a wide variety of proteins are derived from sources that are either animal or plant. Protein-based biopolymers are used to create scaffolds for regenerative tissue engineering applications because of their inherent bioactivity, biocompatibility, and biodegradability. These biopolymers are made into tissue engineering structures, carriers for the delivery of medicinal molecules, emulsifiers, and food packaging materials. Some of the most significant examples of this kind of biopolymer are collagen, albumin, keratin, insulin, hemoglobin, PFF, and others (Nagarajan et al. 2019). Proteins like collagen, gelatin, keratin, sericin, and fibroin are utilized to create a variety of formulations, including films, Pickering emulsions, hydrogels, nanogels, and nanofibers. These formulations were taken into consideration for the regeneration of both hard and soft tissues, including nerve, bone, and cartilage tissues. The extracted proteins are generally very brittle, have poor toughness and elasticity, and have a poor inclination to form films. Thus, by adding reinforcement in the form of synthetic polymers and inorganic nanoparticles, the physical properties of the biopolymer scaffolds must be enhanced. Proteinaceous biopolymers provide a number of benefits, but their uses are restricted by factors including antigenicity and immunogenicity. By carefully enhancing the extraction process to get rid of the antigenic residues, these drawbacks can be eliminated or at least reduced (Reddy et al. 2021).

There are 28 members of the collagen family, and each one has at least one triple-helical domain. Based on how they are dispersed throughout the animal, type I, type II, and type III of collagen can be identified. Along with type I, type II and type III are also typically observed. The skin, tendons, organs, the bone (the main organic component of the bone), and the vasculature are all home to type I and type II, respectively (major collagenous component of cartilage). One property of this protein, which is identified by its unique chemical composition, is the specific amino acid composition of collagen. Collagen, regardless of its origin or form, includes 19 amino acids, including hydroxyproline, which is absent from other proteins. Proline and glycine are present in significant concentrations, and cysteine is absent, which are characteristics of its unusual amino acid composition. Fig. 7 depicts the collagen's hierarchical arrangement. The extracellular matrix is where collagen is deposited, and there, the majority of it forms supramolecular assemblies. Collagen is the protein found in most animal tissues and is found both in soft and hard connective tissues. Natural collagen may be created in a lab setting into highly structured, three-dimensional scaffolds that have great tensile strength and are innately biocompatible, biodegradable, and nontoxic when applied externally. Collagen is the

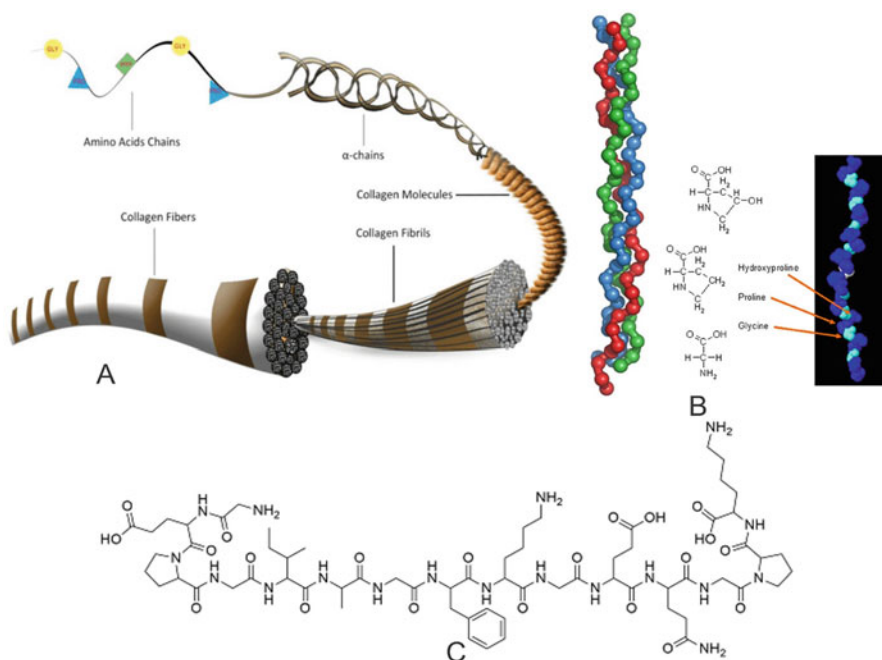


Fig. 7 A schematic illustration of the hierarchical organization of collagen from fibers to amino acid sequence (a). Open and three-dimensional structure of collagen highlighting the unique amino acids hydroxyproline, proline, and glycine (b) and part of a typical amino acid sequence of a collagen chain (c).

chosen material for tissue engineering and wound healing applications because of these characteristics (Mitra et al. 2011; Chattopadhyay and Raines 2014).

In the presence of diluted acids, collagen found in animal skin and bones is thermally denaturalized to create gelatin, a biopolymer. Glycine, proline, and 4-hydroxyproline residues are abundant in gelatin. Gelatin's history dates back many centuries, but it wasn't until the first patent for its manufacture in England in 1754 that its significance was understood. Since then, it has been the preferred material used by all scientists for creating hydrogels at all scales, including nanoscale for drug delivery. Gelatin is now regarded as a food additive by the WHO and is deemed to be entirely safe. Chemically, gelatin is a pure, natural protein that contains 18 different amino acids, including glycine, which makes up 27% of the protein; proline, 16%; valine, 14%; and hydroxyproline, 14%, among others. Gelatin is used as a gelling agent in food, medicine, vitamin capsules, cosmetics, paper, and photographic films, among other notable applications. Probiotics, a mixture of living bacteria and/or yeast that reside in the host body, are also encapsulated in gelatin, either alone or in conjunction with other substances. The use of collagen in food also extends to the pharmaceutical, meat, dairy, dessert, and cosmetic industries as well as beverages and juices (Haug and Draget 2009; Ramos et al. 2016).

Humus-Derived Biopolymers

The “black side” of life, referred to as humus, is created through the interaction between soil organic matter (SOM) and soil organisms. A majority of the benefits humus offers the soil ecosystem are associated with its large surface area for nutrient exchange, capacity for holding onto water, and strong affinity for mineral particles. Humus plays a significant role in the ecosystem by moving organic matter throughout the soil profile and mixing closely with mineral particles. The biological origin of humus and the series of physicochemical processes by which living matter is transformed and recycled within the soil ecosystem to produce a range of chemical compounds, ranging from amino acids (phenylalanine, tyrosine, tryptophan, aspartic acid, arginine, and histidine, to name a few) to large molecules typically categorized as biopolymers, are revealed by thorough examination of humus profiles by microscopic and other spect.? The carbonaceous, polymeric by-products known as humins, which include humic acid, fulvic acid, humin, etc., are generated virtually invariably (Fig. 8). The unregulated cross-polymerization processes of the precursor molecules, such as hydroxymethylfurfural (HMF), levulinic acid (LA), and other reaction intermediates, culminate in the formation of these humins in SOM (Greenland 1971; Aiken et al. 1985).

Bacterial Biopolymers

Nearly all areas of the world are home to bacteria, which are essential to its ecosystems. Some organisms can survive in harsh environments with high pressure and temperatures. In fact, it is thought that there are more bacterial cells in the human body than there are human cells. These tiny, one-celled critters are tiny. Bacteria are efficient cell factories capable of converting carbon and nitrogen sources, and they are capable of producing a wide range of intracellular and extracellular biopolymers, including polysaccharides, polyamides, polyesters, polyphosphates, extracellular DNA, and proteinaceous components. Numerous classes of biopolymers, including polyesters (hydroxy fatty acids linked by ester bonds), polyamides (consisting of amino acids connected by peptide bonds —NH-CO-), polysaccharides (consisting of sugars and/or sugar acids connected by glycosidic (C-O-C) linkages), and polyphosphates (polyPs; composed of inorganic phosphates connected by anhydride bonds), can be produced. Charged or uncharged, non-repeating or repetitive, branched or unbranched, and homopolysaccharides or heteropolysaccharides are all possible characteristics of polysaccharides. Numerous bacteria produce polysaccharides, which they can either exude as free exopolysaccharides or as capsular polysaccharides that are adhered to the cell surface or store inside the cells (like glycogen) (e.g., alginate and cellulose). These biopolymers, which also exhibit remarkable material properties, are home to the most significant virulence factors. Another family of biopolymers produced by bacteria is intracellular cyanophycin, a copolymer of L-aspartic acid and L-arginine that can act as a matrix or capsule for biofilms. It is related to polyamides and poly(amino acid) chains of the same family

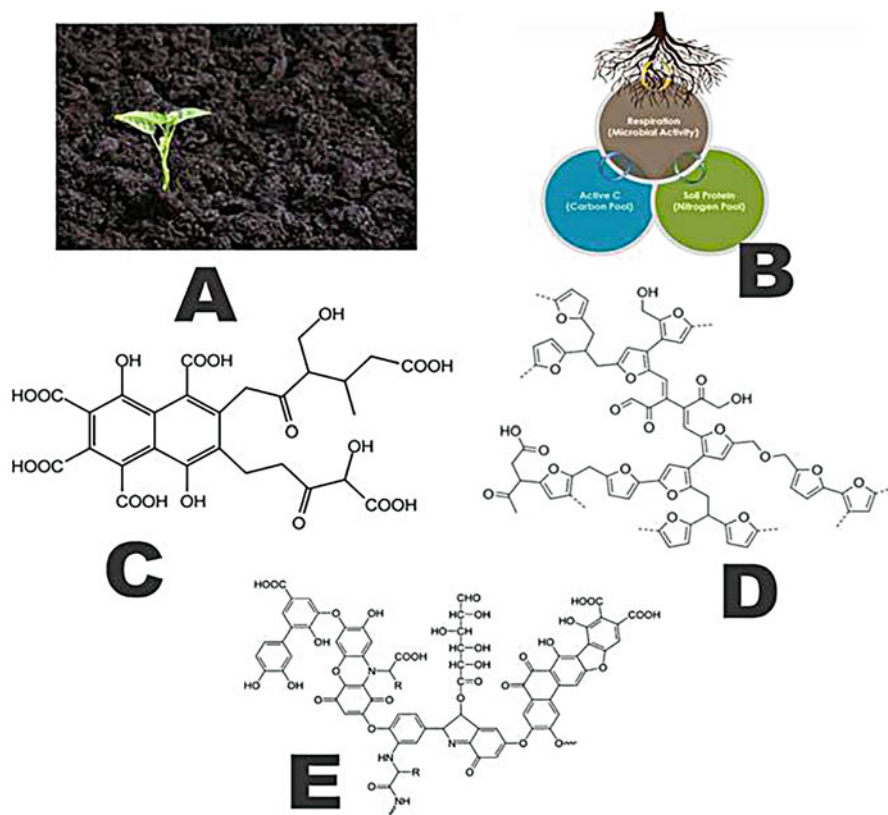


Fig. 8 Scheme showing the humus pool (a), the main chemical constituents such as soil protein (nitrogen pool) and active carbon (carbon pool) microbial activity (respiration) (b), and the certain biopolymers extracted from humus including fulvic acid (c), humin (d), and humic acid (e)

(Fig. 9a). Bioplastics made from bacteria include poly((R)-3-hydroxybutyrate) and other polyhydroxyalkanoates (PHAs). They are hydrophobic spherical inclusions made of linear polyesters that are produced and used as carbon and energy storage (Fig. 9b). Polyphosphates (polyPs) are highly negatively charged polymers of condensed phosphates that are abundant in “high-energy” anhydride linkages. They can include three to several hundred inorganic phosphates. It performs the role of an energy-storing polymer (Fig. 9c). The lifestyle, persistence, viability, growth, stress tolerance, and virulence of bacteria are all impacted by the regulation of cell signalling by polyPs. Other biopolymers, such as extracellular DNA and proteinaceous components, are also produced by bacteria. They are taken into consideration for the creation of bio-based materials in addition to being significant in bacterial parthenogenesis.

A growing amount of attention has been paid to bacterial nanocellulose (BNC) during the past few decades due to its exceptional physical and chemical

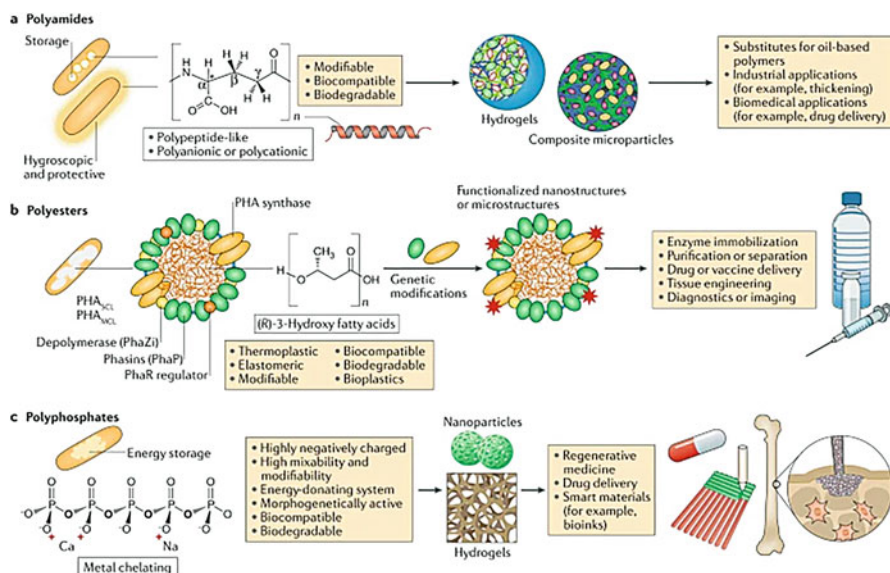


Fig. 9 Bacterial polymer granules of polyamides, polyesters, and polyphosphates have been taken into consideration as alternatives to synthetic polymers made through chemical synthesis that can be used in formulations for industrial, medicinal, pharmaceutical, and cosmetic applications. **(a)** Specific synthetases produce polyamides, which are made of amino acids and synthesized outside of the ribosome. **(b)** Polyhydroxyalkanoates (PHAs), including polyhydroxybutyrate, are hydrophobic spherical inclusions that are produced from (R)-3-hydroxybutyric acid. PHA nanobeads can serve as efficient platforms for tissue engineering, protein purification, medication or vaccine delivery, bioseparation, enzyme immobilization, diagnostics, and imaging. **(c)** Orthophosphates, which range in number from three to hundreds of phosphates, make up polyphosphates. These orthophosphates are joined by phosphoanhydride (P-O-P) bonds. They can be transformed into hydrogels or nanoparticles for a variety of uses and aid in energy storage

characteristics. BNC, often referred to as microbial cellulose, is a fascinating pioneering biomaterial with a distinctive structure and set of features. It has advantages over conventional synthetic fibers, including superior biocompatibility, high mechanical strength, and tailorable surface chemistry, availability everywhere in the globe, biodegradability, and nontoxicity. In comparison with cellulose derived from plants, it also has advantages such as high purity (free of lignin, hemicellulose, and pectin), high crystallinity, high elasticity, high conformability, low density, high specific surface area, high degree of polymerization, excellent permeability, high porosity, and water content. BNC is a highly pure form of cellulose that is created by bacteria from dextrose and other cheap carbon sources with precise metabolic reactions. The bacteria then exude cellulose fibrils that self-assemble in the culture medium (Bäckdahl et al. 2006; Reiniati et al. 2017). It is devoid of additional polymers like lignin, hemicelluloses, and pectin, typical constituents in plant cellulose composites, as well as functional groups like carbonyl and carboxyl added in plant celluloses during the meticulous isolation and purifying procedures (Klemm

et al. 2005). BNCs are made from pure cellulose and have stable network architectures, with average diameters of 20–100 nm and micrometer lengths. To generate bacterial nanocellulose, gram-negative bacteria including *Rhizobium*, *Xanthococcus*, *Gluconacetobacter xylinus*, *Pseudomonas*, *Medusomyces gisevii*, *Azotobacter*, *Aerobacter*, and *Alcaligenes* were combined. This is a new biomaterial that has enormous promise as a biological implant, a material for healing wounds and burns, and scaffolds for tissue regeneration, among other uses.

Challenges and Future Prospects

Therefore, further biopolymer-based research needs to be done in order to close the gaps that now exist by transferring production from a laboratory size to an industrial or commercial scale, achieving the viability and sustainability of completed materials, and putting them on the market as a desirable product. Developing functional materials from molecules and biopolymer research face a number of significant hurdles in the future, which include (1) understanding the crystallization and nucleation mechanisms of pure biopolymers to identify the best bio-additives to enhance crystallization kinetics, (2) streamlining the entire process and creating new (sustainable) techniques for the production of new materials, (3) implementing life cycle assessment related to environmental aspects of biopolymer-based materials, (4) reducing the energy and time consumption of the biopolymer-based materials, (5) energy auditing of the various preparations, (6) how biomimetics can be achieved with various biopolymers and biopolymer-based functional materials, and (7) developing bio-nanocomposites made of nanocrystals or nanofibers derived from renewable resources or agricultural wastes. Despite these multiple significant challenges, we believe that biopolymers and biopolymer-based products will surely enhance people's quality of life in the future through the development of a variety of new functional materials. As a result, to address the present issues and upcoming challenges in creating materials from biopolymers for the human, future research on biopolymer-based materials must take into account various aspects such as numerical models, artificial intelligence (AI), and machine learning (ML)-based algorithms.

Conclusions

Nonetheless, before use on a widespread scale, the development process must integrate the overview of durability, scalability, and real-world efficacy of the materials. Many of these substances are promoted as “green,” “sustainable,” or “eco-friendly” substitutes for conventional petro-based goods. These credentials must, however, pass rigorous testing that examines every stage of the lifetime of each of the products under consideration, from diverse bacterial biopolymers to biomaterials derived from humus, including treatment, discharge, and environmental disposal. Extensive use of biopolymers brings additional challenges to scientists.

Biopolymers exhibit lower end-use properties, crystallization kinetics, and processability than conventional petro-based polymers. Consequently, the major current research effort is to improve mechanical properties, crystallization kinetics, and thermal resistance and barrier properties of biopolymers, which can potentially bring in desired properties for cutting-edge applications.

Cross-References

- ▶ [Amylose–Amylopectin Ratio](#)
- ▶ [Bacterial Nanocellulose \(BNCs\) Supported Inorganic Nanomaterials for Catalytic Applications](#)
- ▶ [Bacterial Nanocellulose from Agro-industrial Wastes](#)
- ▶ [Biomedical Applications of Chitin](#)
- ▶ [Biomolecules from Humus Oil](#)
- ▶ [Biopolymer-Based Nanocomposites for the Treatment of Organic Pollutants from the Synthetic Wastewater](#)
- ▶ [Biopolymers from Marine Prokaryotes](#)
- ▶ [Biopolymers from Renewable Sources](#)
- ▶ [Cellulose Nanocrystals \(CNCs\) Supported Inorganic Nanomaterials for Catalytic Applications](#)
- ▶ [Cosmetic Applications of Albumin](#)
- ▶ [Nanocellulose-Based \(Bio\)composites for Optoelectronic Applications](#)
- ▶ [Nanocellulose from Cladophora](#)
- ▶ [Peptide-Based Biopolymers in Biomedicine and Biotechnology](#)
- ▶ [Recent Approaches Toward Bacterial Nanocellulose \(BNC\) Synthesis](#)
- ▶ [Sustainable Biodegradation and Extraction of Keratin with Its Applications](#)

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Part II

Biopolymers: Synthesis/Extraction



Biopolymers from Renewable Sources

2

Devu P. Kumar, Amrutha S. Nair, Preetha Balakrishnan, and
Sreerag Gopi

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D. P. Kumar · A. S. Nair · P. Balakrishnan · S. Gopi (✉)

Centre for Innovations and Technologies (CIT), ADSO Naturals Private Limited, Bangalore, India

Abstract

Plastic trash has long been recognized as a significant environmental issue throughout the world. Due to their resistance to microbial attack and difficulty in degrading, traditional polymeric substances build up in the environment and are a significant cause of pollution. Due to the problems brought on by synthetic waste, new polymeric materials that are biodegradable and environmentally benign must replace the current ones. Due to their numerous uses in packaging, agriculture, and biomedicine, biodegradable polymers based on renewable resources have received a lot of attention lately. This study seeks to provide an overview of classification, properties, and application areas.

Keywords

Biopolymers · PLA · PHA · pHBA · Renewable resources · Biodegradable

Introduction

The biodegradability, biocompatibility, lack of toxicity, ease of manipulation, and pervasive nature of biopolymers derived from natural sources like plants, microorganisms, and animals have aroused interest in medical applications. Renewable resources are the main sources of biopolymers. The recent growth of nanoscience has revealed a strong preference for the creation of secure and useful nanobiomaterials. It is becoming more and more important to create novel bio-based products and other cutting-edge technologies that can end the global reliance on fossil fuels. To put it simply, bio-based materials are manufactured items made from renewable agricultural and forestry feed stocks. For material applications, a large range of naturally occurring polymers generated from renewable resources is accessible. Some of them, like cellulose and starch, are being used in products, but many others are still unused.

Fossil carbon sources have limited supply, accessibility, and explorability. Over the previous few decades, there has been almost constant growth in oil prices, which peaked in the summer of 2005. Alternative resources and processes will soon replace fossil ones due to the scarcity and high cost of these resources. Because they could assist us in resolving these issues, biotechnological technologies for the production of energy and chemicals have drawn a lot of interest. Approximately 94% of the fossil fuels used today are used to produce energy; the chemical industry uses far less of these resources to produce bulk and fine chemicals.

Biopolymers from plant materials and microbes, which can be developed endlessly, would establish a sustainable industry since they are renewable, carbon neutral, and always sustainable. The effective use of biopolymers and promising new routes with lower carbon footprints resulting from green materials augur well for the design and development of ever-more advanced green materials in the future.

Getting fuels and chemicals from renewable sources is gaining popularity. The problem is that petroleum, the rival resource, is not expensive. Additionally, 50 years

of investment in petroleum processing have made it possible to efficiently transform petroleum into an incredible variety of products. As a result, in low-value applications, items obtained from renewable resources have had little success competing with those derived from petroleum (e.g., fuels and simple chemicals). Even for intermediate-value goods, renewables have had trouble competing with petrochemicals (e.g., specialty chemicals and polymers). For instance, it is possible to create water-soluble polyacrylamides that have a variety of functional characteristics that can be tuned for certain purposes. If cellulose is heavily chemically altered, some of these qualities can also be obtained with this natural polymer.

For a variety of biomedical, agricultural, and ecological goals, it is desirable to have a biodegradable plastic that will disintegrate in the physiological environment or by microbial action in the soil. A lot of work has gone into improving the biodegradability of plastics, with the major focus on the development of new biodegradable polymers, modifications to natural and synthetic polymers, and composites made of biodegradable polymers. Attention has been paid to biopolymers as beginning materials in the hunt for biodegradable polymers based on renewable resources. One of the main biopolymers in crops is plant protein. It is a biopolymer that is renewable and biodegradable. The use of plant proteins as a material is not very common. Casein, zein, and other types of plant proteins have been used to make fibers and polymers.

An organic polymer that is generated by living creatures is referred to as a "biopolymer." Recent developments in our understanding of important biosynthetic processes and the options to control or modify these pathways through genetic changes have led to the investigation of new possible applications for polymers generated from renewable resources. Biopolymers are composed of numerous polysaccharides, proteins, lipids, polyphenols, and specialized polymers made by bacteria, fungus, plants, and humans. For instance, environmental compatibility would be enhanced because using it wouldn't result in any additional environmental duties in. The use of renewable resources encourages the production of more nonrenewable petrochemicals. The improvement of biopolymers using polymers derived from renewable and sustainable resources is a modern and promising way to minimize greenhouse gasoline and hazardous emissions, as properly as strength consumption and the use of nonrenewable resources.

Synthetic polymer-based materials are not biodegradable and are frequently thrown carelessly. Environmental problems have resulted from the massive development of conventional plastics created from synthetic polymers derived from petroleum that cannot break down in a composting or landfill environment. A creative and practical solution to reduce toxic and greenhouse gas emissions, energy demand, and the use of nonrenewable resources is the development of biopolymers employing polymers generated from sustainable and renewable resources.

There are many benefits to using naturally occurring polymers in materials. The use of biodegradable materials for packaging, agriculture, medicine, and other applications has gained popularity recently. As an alternative to the synthetic polymeric materials now used, a variety of biopolymer mixes should be used. These include starch, chitosan, alginate, gelatin, PLA, PHAs, and other common and useful biopolymers.

One significant advantage of biopolymers is their complete success at biodegradation at accelerated rates, decomposing in a controlled amount of time under the enzymatic action of microorganisms into simple molecules. Under certain environmental conditions, polymeric materials made from renewable resources may be biodegradable or compostable. They are labeled based on the production process or the source:

- Polymers like proteins and polysaccharides that can be quickly removed from biomass.
- Standard chemical synthesis is used to create polymers, beginning with sustainable bio-based monomers like polylactic acid (PLA).
- Microorganism- or genetically modified-derived polymers such bacterial cellulose and polyhydroxyalkanoates.

Consumer expectations and technological advancement are increasing the demands on the world's resources, which is creating serious problems with material availability and environmental sustainability. Biofiber composites have seen a remarkable metamorphosis during the past few decades. As novel compositions and technologies have been extensively explored, created, and then used, these materials have gotten steadily more sufficient. Biocomposites have gained major importance as a result of the oil crisis and have developed into engineering materials with a very diverse set of features. However, like all materials, they are continually subject to market competition on a worldwide scale, which calls for ongoing study. The days of simply combining plastics with natural waste fillers and describing the major characteristics of the resulting mixture are over.

Nano-catalysts based on biopolymers can be made more stable and effective by immobilizing them on reactive groups with unique properties. The abundance of size, structure, and molecular chains in low-cost biopolymers such polysaccharides makes them desirable candidates for stabilization and immobilization as well as the reduction of NPs. Biodegradable materials are increasingly being used in packaging, agriculture, medicine, and other fields in recent years. Recently, a wide range of applications for biopolymers have been identified, from bioplastic additives and mixes to consumer goods, research products, and personal hygiene items – all of which offer the benefit of environmentally friendly decomposition. The functionality of biopolymers in a variety of applications is influenced by the choice of the biopolymer, its intended use (emulsion and foam surface recreation or viscosity forming bulk shape or gelation), the process of structure development, and its interaction with any additional formulated matter.

For a variety of biomedical, agricultural, and ecological goals, it is desirable to have a biodegradable plastic that will disintegrate in the physiological environment or by microbial action in the soil. A lot of work has gone into improving the biodegradability of plastics, with the major focus on the development of new biodegradable polymers, modifications to natural and synthetic polymers, and composites made of biodegradable polymers. Attention has been paid to biopolymers as beginning materials in the hunt for biodegradable polymers based on renewable

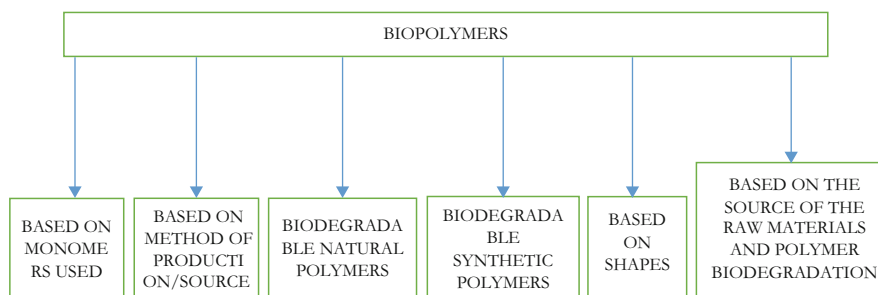
resources. One of the main biopolymers in crops is plant protein. It is a biopolymer that is renewable and biodegradable.

In addition to their usage in construction materials and for specialized purposes, biopolymers are widely used as packaging materials. For this reason, cellulose chemical compounds made from plant biomass have been employed for many years. For use as packaging materials, a number of biopolymers made using biotechnological techniques have been created. It might be advantageous for the environment if biodegradable polymers made from renewable resources are available. The biotechnological production of non-biodegradable biopolymers for applications that require them hasn't gotten much attention yet, despite the enormous demand for persistent materials. Without the availability of synthetic durable polymers like polyethylene, polystyrene, or polyvinyl chloride, it would be impossible to build modern homes, cars, or other significant advancements.

Classification of Biopolymers

In the modern day, using compounds that disintegrate easily in the environment plays a significant role. Biopolymers are one of these materials; they are made utilizing living creatures. The monomeric components of biopolymers include nucleotides, amino acids, and sugars. Among the chemicals that make up biopolymers include cellulose, starch, chitin, proteins, peptides, DNA, and RNA. Biopolymers and their derivatives exhibit the qualities of an excellent and are becoming increasingly important for many activities. A variety of biopolymer mixtures could replace some of the synthetic polymeric materials currently in use. Starch, chitosan, alginate, gelatin, PLA, PHAs, and other commonly used and prospective biopolymers are also listed. The primary resource for developing renewable and biodegradable polymer materials to replace petrochemicals is herbal polymers generated from agricultural products. New potential applications for the utilization of renewable polymers are being researched thanks to improvements in our understanding of major biosynthetic processes and our capacity to control or customize those pathways through genetic changes. Of their herbal surroundings, evidently going on polymers serve an extensive variety of duties. Membranes, intracellular communication, cellular surface reput, mobile wall construction, capsular layers or protective barriers around cells, emulsifiers, adhesives, and water, nutrition, and steel sequestration for cells are all places where polysaccharides are hidden. Proteins serve as building blocks, catalysts, cell characteristic regulators, adhesives, and reputation-building elements. Biopolymers have created an excellent competition with their fossil gasoline-based totally equivalents in phrases of useful characteristics and manufacturing charges. While the cost of producing biopolymers could be very low, continuous efforts are made to enhance their purposeful residences.

Biopolymers are divided into classes: biodegradable and non-biodegradable. Some other classification is based on their source of starting place: herbal resources (plant life, animals, and microorganisms) or fossil fuels. While measured in terms of usability, non-degradable biobased biopolymers outperform biobased degradable

Table 1 Biopolymers and their classifications

biopolymers in several functional domains. Biopolymers are classed as elastomers, thermosets, or thermoplastics based totally on their potential to respond to warmness situations. In evaluation to biobased thermoplastics, biobased thermosets have lately sparked more interest in generating components, even as elastomers have inspired many scientists and scholars to produce revolutionary substances with their personal set of appropriate capabilities. Finally, biopolymers are classified into three groups based on their composition: blends, laminates, and composites. Classification of biopolymers are divided in Table 1.

Recently, microbially produced biopolymers including polyhydroxy alkonates (PHAs) polymers have gotten quite a few hobby. Using renewable materials is turning into extra essential, and the world's essential industries and producers are seeking out ways to update diminishing petrochemical-based totally feedstock with composites crafted from herbal fibers and biopolymers.

Based on Monomers Used

Based totally on monomers used, biopolymers can be categorized into three: polysaccharides (from sugar), proteins (from amino acids), and nucleic acid (from nucleotides). Most biopolymers' monomers are composed of repeating molecules of either nucleic acids or nucleotides, amino acids, proteins, or saccharides generated from sugars.

Polysaccharides from Sugars

Amylose/Amylopectin is a kind of starch. Relying on the plant species, the ratios of amylose and amylopectin range (normally about 25–30% amylose in corn starch). Starch is crucial as a biopolymer resource because of its low cost, availability as an agricultural surplus uncooked fabric (approximately one billion bushels/12 months carryover in the), and thermal processability using standard plastics processing equipment. Because of the hydrophilic nature of starch, water management is crucial both during and after processing.

Even though the starch percentage in early starch blends was often around 10% and non-biodegradable additions like polyethylene served as the main component, starch blends were created to capitalize on the biodegradable properties of starch. Some of those mixtures contain a car-oxidant that interacts with steel salts in diverse environments or soils, producing peroxide radicals that catalyze the breakdown (depolymerization) of the mixture's polymers, including polyethylene.

The starch is handled with a silane coupling agent to make it compatible with polyethylene, and an unsaturated ester, such as soya or maize oil, can be used as the car-oxidant. Temperature triggers based on a composting habitual have been considered with the aid of different era.

Cellulose. Vegetation and microorganisms produce cellulose, that's a linear polymer made of p-1,4-related n-glucose (e.g., *Acetobacter xylinum*). Cellulose is the most abundant naturally occurring polymer in the biosphere. The cellulase enzyme complex catalyzes the formation of cellobiose and glucose during regular cell biochemical cycles, which causes mineralization. Due to the excessive hydrogen bonding, cellulose cannot be thermally handled and need to go through thermal disintegration earlier than melting; however, it may be solvent solid. The lyotropic mesophase is discovered in cellulose and numerous cellulose derivatives.

In addition to being used in paper and paper products, membranes and films, fabric, food, and medicine, cellulose also serves other purposes. The speaker diaphragms in headphones are made from cellulose derived from bacteria because it has a better crystallinity and smaller diameter fibrils than cellulose derived from plants. Cellophane is one of the most prevalent types of cellulose used in packaging. Due to its transparency and oil barrier features, it is utilized as laminated wrappers for more than a few food. To enhance moisture barrier features, cellophane is typically covered with nitrocellulose or poly (vinyl chloride), despite the fact that those coatings are not biodegradable.

Cellulose is water insoluble in its local circumstance, making it difficult to convert into films or coatings. Derivatization reactions boom water solubility through forming carboxymethyl cellulose, methyl cellulose, hydroxy propyl cellulose, and other systems. Mineralization rates are slowed by crosslinking. With increasing levels of substitution, both steric difficulty and a lower hydrophilicity is liable for the lower degradation rates. Nitrocellulose (containing di- or trinitro substitutions consistent with monomer), in addition to cellulose tripropionate and cellulose tributurate, is all proof against microbial assault.

Pectins. Pectins are structural polysaccharides that may be isolated from plant cells and consist primarily of α -1,4-n-galacturonic acid with varying degrees of methyl esterification (middle lamella). Pectin is a viscous substance that dissolves in water. Citrus peels and apple pomace are the maximum common business resources of pectin. In the presence of calcium ions, low methoxyl pectin forms a gel in the presence of calcium ions and is used as a coating and additive in the food zone.

Proteins from Amino Acids

Soy, Zein, Wheat Gluten, Casein, and Serum Albumin are a number of the proteins discovered in soy. Soy, zein (an alcohol-soluble protein extract from corn gluten),

and wheat gluten are all components of vegetables. A protein blend called casein is produced from the milk of mammals. Blood plasma or milk from mammals are the sources of serum albumin. Among other things, casein is used to make glue, fortify flour, and create microbiological media. The genes encoding those proteins have been cloned and produced, and serum albumin is hired as a therapeutic protein for blood diseases.

Collagen/Gelatin. Collagen is the most ample protein inside the animal country and an enormous structural thing in animal tissue. Mammalian skin, bone, and connective tissues all contain collagen. Collagen has an essential shape that has the glycine-X-Y trimer repeat, where X and Y are usually proline or hydroxyproline. The movement of some of protease enzymes can depolymerize collagen, similar to every other protein. Water or solvent processes generate collagen films, which are sometimes crosslinked. Those fibers are broadly speaking employed in clinical programs and as a casing fabric in meals. Collagen has been used in pharmaceuticals for tissue and nerve repair, wound dressings, medication delivery, ophthalmology, vascular grafts, and sutures of many types, including sheet, tube, sponge, and powder.

Gelatin. It is an animal by-product made by partially hydrolyzing collagen and then extracting it with hot water. Gelatin is biodegradable and without difficulty crosslinked. Chemical processes consisting of glutaraldehyde, carbodiimide, acyl azide, isocyanates, and different chemistries may be used to crosslink substances. In widespread, gelatin has a molecular weight variety of 15,000 to 250,000. The majority of natural solvents are insoluble in gelatin, but water, acetic acid, and polyhydric alcohols are soluble. With the addition of plasticizers, gelatin can also be used in textiles in the food, pharmaceutical, and photography sectors. Biodegradability of gelatin and poly(ethyl acrylate) graft copolymers in movie shape has been discovered.

Silks. Many animals, including insects and spiders, create silks, which are large-molecular-weight proteins. Many silks contain a secondary structure known as a “sheet,” which is maintained via hydrophobic interactions between sheets and interchain hydrogen bonds. Many silks include high levels of short-chain amino acids including glycine, alanine, and serine, which can range from 65% to 85%.

Method of Production and Source

Underneath certain environmental conditions, polymeric materials made from renewable assets can be biodegradable or compostable. They are classified primarily based on the method of production or the source of formation.

Polymers Directly Extracted from Biomass such as Polysaccharides and Proteins

Because of its abundance, low fee, and renewability, starch is a probably promising biodegradable material. Furthermore, starch’s utility in the plastics industry can assist to lessen reliance on synthetic polymers. Amylose and amylopectin are

typically the two types of polymers that make up the heterogeneous fabric known as starch. It has been demonstrated that it is a heterogeneous material even if the shape of it has not yet been fully investigated. Even though starch is a commonplace aspect in biodegradable plastics, natural starch-primarily based films have poor mechanical traits. The most crucial polysaccharide is starch, which is abundant in nature and comparatively reasonably priced. Herbal starch is to be had in granular shape and has been used as a filler in polymers, but it may additionally be thermoplasticized and handled with conventional plastic processing techniques along with extrusion, foaming, and film blowing. The hydrophilic function of starch is its predominant disadvantage, proscribing its software in excessive-moisture situations. Starch can be used to create PLA composites, and it can also be mixed with other biodegradable polymers and derivatives to create fully degradable biomaterials, such as polyvinyl alcohol, poly-hydroxybutyric acid, polycaprolactone, and chitosan. Chitosan has become one of the most well-liked biopolymers for the creation of drug delivery systems for a range of packaging due to its promising properties, which include high biocompatibility, remarkable biodegradability, low toxicity, plentiful availability, and low cost of coffee manufacture. Chitosan is a biopolymer made via the de-acetylation of chitin, the second one maximum universal biopolymer after cellulose in nature.

Chitin can be found inside the exoskeletons of bugs, crabs, shrimp, lobsters, and sure fungal cellular walls. It is economically feasible to supply chitosan from crustacean shells, which can be discarded by using the seafood enterprise. Due to its film forming competencies, chitosan has been recommended as an appropriate meals packaging cloth, specifically for fit for human consumption movies and coatings. However, one of the most enormous hazards of chitosan-based composites is their low mechanical strength. By causing inter- or intramolecular crosslinking in the polymer matrix, chitosan is frequently combined with other polymers or crosslinked to improve their usable qualities.

Cellulose is a crucial and charming biopolymer, as well as almost infinite and sustainable herbal polymeric raw cloth with applications in both industry and ordinary existence. Within the cell partitions of superior flowers are microfibrils with a helical organization on multiple levels, crystalline domains (domains with ordering high cellulose chains), and amorphous (section fields distorted, twisted, and deformed).

A few of cellulose's intriguing properties are its mechanical toughness, hydrophilicity, biocompatibility, biodegradability, relative thermostability, excessive sorption capacity, and modifiable visual appearance. Cellulose is broadly utilized in a diffusion of programs. Based on cellulose, they offer numerous benefits (specially their wise responses to environmental stimuli) and may be used in a variety of situations. Every 12 months, around 51,011 metric tonnes of cellulose are produced. Lamentably, just 2% of the full is recovered industrially. Cellulose's first rate qualities permit it for use in an extensive variety of fields.

Collagen is extensively hired as a biomaterial in a huge variety of biomedical applications. The most prevalent proteins found in the extracellular matrix of vertebrates are collagens. The most abundant type of collagen found in animal

hides and skins is type I collagen, which is also the most crucial structural component of tendon, bone, and connective tissue. Collagen is seen as fibrils, which are responsible for the structural support and mechanical function of connective tissues. Because of their natural structure, biodegradability, and biocompatibility, collagens have a wide range of applications as biomaterials in tissue engineering, wound healing, drug transporters, and cosmetics. For instance, fibril-forming collagens serve as a scaffold for cell attachment and migration while also supplying mechanical properties.

A key task in fabric studies is the creation of suitable modification strategies to enhance the properties of natural polymers. One example is the creation of natural polymers based on wheat gluten or wheat protein. One of the most affordable plant proteins is wheat protein, often known as wheat gluten, which is derived from the second-largest cereal crop (after maize). Super viscoelastic, tensile, and gasoline barrier properties describe the properties of wheat proteins or gluten. Wheat gluten, a derivative of the starch industry with an excessive protein content (>75 wt%), may be considered best for a variety of applications due to its suitable thermoplastic properties, processability, and high-quality biodegradability.

Polymers Produced by Classical Chemical Synthesis Starting from Renewable Bio-based Monomers such as Polylactic Acid (PLA)

Because of its renewability, biodegradability, biocompatibility, properly thermo-mechanical traits, and occasional fee, polylactide (PLA) is the quality polymeric replacement for various petropolymers inside the context of ecologically friendly procedures and products. To begin with, the general public of its applications were in the biomedical area and quick-time period usage which includes packaging, because to PLA's biodegradable qualities. Due to the depletion of petroleum resources, PLA is increasingly being considered as a valuable bio-sourced polymer substitute in long-term applications such as automotive and electronics. PLA is a more environmentally friendly material with more residences for usage inside the human body (nontoxicity). There are numerous ways to create lactic acid polymers, which results in products with a wide range of chemical and mechanical properties.

Due to their excellent biocompatibility and mechanical properties, PLA and related copolymers are being used more frequently in tissue engineering to restore function to injured tissues. It's a biodegradable polymer with an extensive range of applications that may be changed into unique resin grades for processing into a spread of merchandise. Lactic acid-primarily based polymers (PLA) are one of the maximum promising classes of renewable polymers. PLA has various advantages over petroleum-based polymers which might be usually utilized in packaging: (i) transparency, which is commonly defined as visible light transmission in the 540–560 nm range and is somewhat higher than that of poly (ethylene terephthalate) (pet) and poly (styrene) (playstation); (ii) degradation in biological settings like compost or soil; (iii) biocompatibility: because PLA has been shown to be biocompatible and decompose into non-toxic components, the Food and Drug Administration has approved its use for implantation inside the human body (FDA); (iv) processability: mild processing is the primary way of transforming PLA. Due

to its glass transition temperature (T_g), which is close to 55 °C, PLA is brittle and hard at ambient temperature. PLA is a glassy polymer with a negligible break elongation (commonly less than 10%). In an effort to increase their ductility, a significant number of experiments have been done to modify the properties of PLA-based materials by plasticization and combining with various polymers.

Polymers Produced by Microorganism or Genetically Modified Bacteria such as Polyhydroxyalkonates, Bacterial Cellulose

Polyesters at the moment are widely hired as fibers and films in a variety of applications, and plastic waste control has nowadays emerged as a primary environmental problem. Many microorganisms produce polyhydroxyalkanoate (PHA), an environmentally friendly polymeric substance that can be become movies and fibers from renewable carbon sources. PHA is likewise a terrific material for making resorbable scientific gadgets such sutures, meshes, implants, and tissue engineering scaffolds, consistent with the findings. Under nutrient unbalanced situations such as nitrogen, phosphorous, or oxygen predicament, a huge variety of bacteria can amass those polymers as a carbon and power storage fabric. Greater than three hundred species of Gram-high-quality and Gram-negative microorganism, as well as an extensive spectrum of archaea, use them as a carbon/electricity storage. PHAs may be made from renewable primary materials and are destroyed evidently by microorganisms in the surroundings, permitting carbon dioxide and natural compound recycling and offering a climate exchange buffer. PHAs can be made from renewable basic materials and are destroyed certainly via microorganisms inside the surroundings, permitting carbon dioxide and organic compound recycling and providing a climate trade buffer. Extra-cellular PHA depolymerase launched via microorganisms in various herbal habitats is chargeable for biodegradation of PHA material. In the meanwhile, new research efforts are being made to generate PHAs in transgenic flora. Polyhydroxybutyrate (PHB) and poly (hydroxybutyratecohydroxyvalerate) (PHBV) are the maximum famous polymers of the polyhydroxyalkanoate family. Polyhydroxybutyrate (PHB) is a polyester from the PHA circle of relatives this is saved intra-cellular by means of an extensive range of microorganisms as an organic carbon and chemical power source.

PHB is a biodegradable thermoplastic polyester which is much like many traditional petroleum-primarily based polymers already in use. It also offers some extra blessings, together with being biocompatible and being capable of be created from a renewable raw material in a sustainable method from a monetary and environmental perspective.

Bacterial cellulose (BC) is a fascinating and renewable herbal nanomaterial with suitable functions which include exceptional mechanical features, porosity, water absorbency, moldability, biodegradability, and excessive biological affinity. Intensive research and exploration on BC nanomaterials has generally centered on their biosynthetic procedure if you want to attain low-cost education and application in clinical, meals, advanced acoustic diaphragms, and other fields over the last few many years. Bacterial cellulose has the equal molecular method as plant cellulose; however, it carries three-dimensional porous network architectures which can be specific and complex.

Bacterial cellulose has several advantages over plant cellulose, which include (i) loss of lignin and hemicellulose, making it an exceedingly pure source of cellulose; (ii) polymerization with a high degree of crystallinity (60–70%), ensuing in excessive young's modulus at room and accelerated temperatures; (iii) extremely excessive waterholding potential, as much as 100 times its selfweight; (iv) splendid biodegradability and organic affinity.

Numerous change procedures were investigated to see if extra features may be delivered to BC. In recent years, there was a boom in international hobby in phrases of extensive scientific inquiry and expanded efforts to place BC materials to practical use. The yearly guide activity on BC is growing (also referred to as microbial cellulose or bacterial nanocellulose). In latest years, research has targeted on the investigation and alertness of BC in purposeful materials, and a growing variety of papers have been published in this challenge. Functional BC-based nanomaterials are particularly appealing because they permit the production of substances with better or novel competencies via combining numerous factors and leveraging synergistic effects like as electrical, optical, magnetic, catalytic, and other capabilities.

Biodegradable Natural Polymers

Biodegradability, as well as the ability to support the regeneration of new tissue without causing irritation should be recommended. Polymers as biological entities, are classified into 3 types (i) polysaccharides, (ii) proteins, and (iii) polyesters. Natural polymers can be generated through fermentation of microorganisms or in vitro manufacture by enzymatic techniques, both of which are based on biotechnology advances.

Sugar-Based Biopolymers

Blowing, vacuum forming injection, and extrusion are all methods for making sugar-based biopolymers. Lactic acid polymers (Polyactides) are made from lactose (milk sugar) derived from maize, potatoes, wheat, and sugar beet. Polyactides, which are made through vacuum forming, blowing, and injection molding, are water resistant.

Starch-Based Polymers

Starch-based biopolymers are a natural polymer that can be generated from plants such as wheat, tapioca, potatoes, and maize. This substance is stored as carbohydrates in plant tissues. It is made up of glucose and is made by melting starch. Animal tissues do not contain this polymer. Dextran is a category of low-molecular-weight carbohydrates generated by starch hydrolysis and enzymatically synthesized by immobilized *Enterococcus faecalis* Esawy dextranucrase onto biopolymer carrier.

Cellulose-Based Polymers

Cigarettes, CDs, and sweets are all packed with cellulose-based biopolymers. This polymer is made up of glucose and can be found in natural resources such as cotton, wood, wheat, and maize cellular walls.

Biodegradable Synthetic Polymers

Petroleum is used to make biopolymers based on synthetic chemicals, such as aliphatic aromatic copolyesters, which are biodegradable polymers. Despite the fact that they are made of synthetic materials, they are entirely compostable and biodegradable.

Polyglycolide Acid (PGA)

It's a crystalline polymer with a crystallinity of 45–55%. It also has a high tensile modulus but is poorly soluble in organic solvents (Fatland et al. 2005). Poly (glycolic acid) and poly (lactic acid) are the most extensively utilized synthetic biodegradable polymers at the moment. Because of its superior fiber forming capabilities, polyglycolide was initially studied for use in making resorbable sutures. The citric acid cycle breaks down polyglycolides into glycine or converts them to carbon dioxide and water.

Poly lactide Acid (PLA)

It's a crystalline polymer (37% crystallinity), just like polyglycolide. It has a glass transition temperature of 60–65 degrees Celsius and a melting temperature of 175 °C. In comparison to polyglycolide, poly (L-lactide) degrades slowly. L-lactide (LPLA) homopolymer is a semicrystalline polymer with high tensile strength, minimal elongation, and a high modulus, making it appropriate for orthopedic fixation and sutures with diverse applications such as load-bearing.

Polycaprolactone

A semicrystalline polymer with high polycaprolactone solubility and a low melting point (59–64 °C) can encourage biomaterial development. Due to the short half-life of bioactive compounds, effective transportation of bioactive agents such as drugs, enzymes, and other bioactive agents is required. Starch-poly-epsilon-caprolacton microparticles were then used in a variety of applications, including medication delivery and tissue engineering. In addition, these microparticles were generated using an emulsion solvent extraction/evaporation process.

Polyurethane

The polycondensation reaction of diisocyanates with alcohols and/or amines is used to make polyurethanes. Bio-stable polyurethanes and poly (ether urethanes) are intensively explored for the future view in medical implanting, mostly for cardiovascular disorders such as pacemakers and vascular grafts. Polyurethane is biocompatible and used in a wide range of medical devices due to its flexibility and mechanical strength. Polyurethane can connect to membranes and does not easily elicit immunological reactions, hence it is biocompatible. The Knauth team, who work on prosthetic skin for premature neonates, has also produced a semipermeable membrane.

Poly (ortho esters)

Poly (ortho esters) is a biodegradable polymer that has gained popularity in recent years. Because of its hydrophobic qualities, poly (ortho esters) erode slowly in water

conditions. Poly (ortho esters) are distinguished not only by their surface erosion mechanism but also by their rate of degradation, pH sensitivity, and flexibility in glass transition temperatures, which can be manipulated by altering chain diol levels.

Based on Source of the Raw Materials and Polymer Biodegradation

The definition of a “biopolymer” (or “bio plastic”) is based on two basic criteria: (1) the raw material source and (2) polymer biodegradation.

Biopolymers Made from Renewable Raw Materials (bio-based), and Being Biodegradable

These polymers can be made chemically from biological starting elements or created by biological systems (microorganisms, animals, and plants) (e.g., corn, sugar, starch). Biodegradable bio-based biopolymers include (1) synthetic polymers made from renewable resources, such as poly (lactic acid) (PLA); (2) biopolymers made by microorganisms, such as PHAs; and (3) naturally occurring biopolymers, such as starch or proteins. Natural polymers are defined as those that are biosynthesized through various pathways in the biosphere. Starch and PHAs are the most often utilized bio-based biodegradable polymers.

Biopolymers Made from Renewable Raw Materials (bio-based), and Not Being Biodegradable

These biopolymers are nonbiodegradable and can be made from biomass or renewable resources. (1) Renewable resource synthetic polymers, such as specific polyamides from castor oil (polyamide 11), specific polyesters based on bio propanediol, bio polyethylene (bio-LDPE, bio-HDPE), bio polypropylene (bio-PP), or bio poly (vinyl chloride) (bio-PVC) based on bioethanol (e.g., from sugarcane), etc.; (2) naturally occurring biopolymers, such as natural rubber or amber.

Based on Shape

Biopolymers have been classified by the shape into many types. These type include disks, beads, thin films (membrane), and nanoparticles.

Disk

In the literature, gel discs are frequently employed. Researchers commonly generate a single film of gel using the casting process, such as a Petri plate, and then cut it into discs with cork borers. Previously, a novel equipment called parallel plates are devised that allows them to make several uniform in one step with high accuracy.

Beads

Gel beads are commonly used in industry because they have the largest surface area and may be made using a variety of procedures, including the interphase technique,

ionic gelation methods, dripping method, and the Innotech encapsulator. The Innotech Encapsulator has a high bead production rate (50–3000 beads per second, depending on bead size and encapsulation product mixture viscosity), making it ideal for industrial scale production.

Thin Films (Membranes)

Polymeric membranes are made by dissolving polymer materials in their solvent, then casting, washing, and drying them. Sakurai's study team examines the domain structure and concludes that polymeric materials alter the intracellular concentration of calcium ions, a second messenger in the transmission of biological signals or an enzyme cofactor in the coagulation system. It was also reported in the study that surface grafting of polyoxyethylene chains can significantly improve the membrane from nonthrombogenicity of a PEUU.

Nanoparticles

Nanoparticles are solid particles with a diameter of less than one micron that can be utilized to nanoencapsulate bioactive substances. Depending on the method of preparation, nanoparticles, nanospheres, or nanocapsules can be obtained. Biodegradable polymeric nanoparticles can be made from proteins (such as gelatin and milk proteins), polysaccharides (such as chitosan, sodium alginate, and starch), and synthetic polymers (such as chitosan, sodium alginate, and starch). Various ways for creating nanoparticles from natural materials have recently been used.

Production of Renewable Polymers from Crop Plants

Plants produce a variety of biopolymers which have several functions such as protection of structural integrity, carbon storage, and protection toward pathogens. Several of these natural polymers are used by human beings as food and materials, and more and more as a strength carrier. Plant biopolymers are used as substances in wide applications, such as plastics and elastomers, due to the depletion in resources and climatic changes. Biopolymers have the ability to compete with petrochemical plastics used in consumer goods (e.g., polyethylene and polypropylene) can be a good targets for low-value high-volume biopolymers produced in target proteins are known as fibrous proteins, that are non-ribosomally produced poly-amino acids such as cyanophycin, and plant proteins acquired as co-products of starch, vegetable oil or biofuels (van Beilen and Poirier 2008).

Proteins

In past few years proteins derived from starch or oil crops have been converted to different materials. Through cross-linking with glutaraldehyde, formaldehyde, or other chemicals, plastics can be produced from zein, soy protein, and wheat gluten.

Fibrous proteins include repeated short unit of amino acids and considered as difficult to block co-polymers with special strength to weight, elastic, or adhesive properties. Elastin, wheat glutenin, mussel adhesive proteins, collagen, silk, keratin, and resilin are the examples for fibrous proteins. By changing the linker elements between the repeat sequences, a tremendous combinatorial range was obtained (van Beilen and Poirier 2008).

Non-ribosomally produced polyamino acids: Cyanophycin is a nitrogen storage polymer synthesized with the aid of large range of cyanobacteria. It is synthesized through the enzyme cyanophycin synthetase without the involvement of ribosomes, and deposited as granules. It consists of polyaspartate spine with arginine aspect chains that are connected by using their β -amino group with the α -carboxy group of each aspartate. Although cyanophycin is not a polymer with appropriate properties, it is oftenly used as source of polyaspartate, can change the chemically synthesized fabric and used as a gorgeous adsorbant or antiscalant different poly-amino acids, such as polylysine and polyglutamate, currently they are used in many attainable non-food applications ranging from hydrogels, biochip coatings and drug carriers to cryoprotectants. The genes encoding their synthesis machinery have been cloned and are available for expression in plants (van Beilen and Poirier 2008).

Natural Rubber

Natural rubber is the most important organic material used in non-food applications. it is a polymer composed of isoprene units linked collectively in a 1,4- cis configuration. Natural rubber is formed by using the rubber tree consisting of polyisoprene.

Because of its structure, molecular weight and poorly described components such as proteins, lipids, carbohydrates and minerals natural rubber has unique properties. These residences consist of resilience, elasticity, and abrasion and have a great impact on resistance, efficient heat dispersion and malleability at cold temperatures (van Beilen and Poirier 2008). Synthetic rubbers, such as styrene–butadiene or acrylonitrile–butadiene co-polymers, are commercially produced that are comparable to the herbal rubber. The exceptional residences of natural rubber are irreplaceable for many applications, such as heavy-duty tires for trucks, buses, and airplanes, also latex merchandise for clinical applications. While some distinctive vegetation can produce different varieties of polyisoprenes, but none of them have cloth homes or value similar to Hevea rubber.

Degradation of natural rubber by way of the usage of fungus has been reported. Rubber is used substantially all through the array of materials science functions and in medical fields. Rubber polymers containing polyisoprene (natural rubber and artificial polyisoprene) had been biodegraded by using fungi such as *Penicillium* and the Actinomycete *Nocardia* sp. Recently, a rubber degrading enzyme used to be isolated from the extracellular subculture medium of *Xanthomonas* sp. and crude fractions had been successful of depolymerizing herbal rubber (van Beilen and Poirier 2008) (Fig. 1).

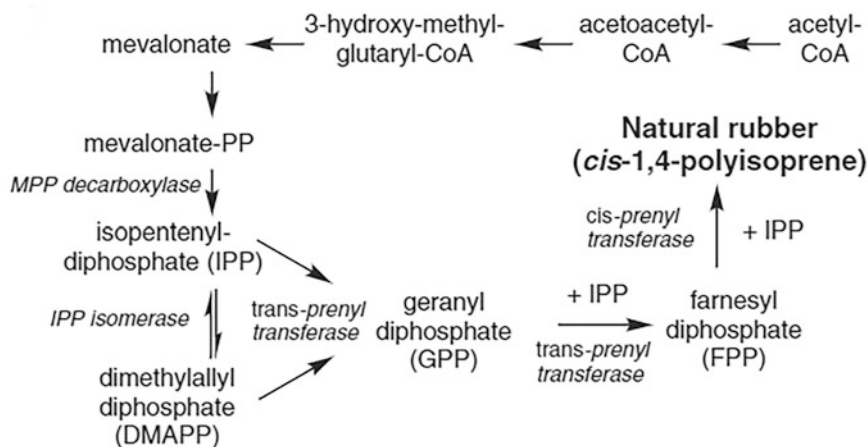


Fig. 1 Schematic pathway for rubber biosynthesis from isopentenyl diphosphate (IPP). A straight side branch of the common isoprenoid route is used to create natural rubber, with the crucial step 3-hydroxy-methyl-glutaryl-CoA coming from acetyl-CoA via the main mevalonic acid pathway. IPP isomerase changes IPP into dimethylallyl diphosphate (DMAPP). Trans-prenyltransferases then combine IPP with DMAPP in a series of steps to create geranyl diphosphate (GPP) and farnesyl diphosphate (FPP). A rubber transferase, also known as cis-prenyltransferase or rubber polymerase, catalyzes the cis-1,4-polymerization using the non-allylic IPP as substrate

Polyhydroxyalkanoates

Numerous bacteria make polyhydroxyalkanoates (PHAs), which are biological polyesters that operate as granule-forming, osmotically inert carbon and energy storage molecules. The majority of naturally occurring PHAs are made up of 3-hydroxy fatty acids with chains ranging from 4 to 16 carbons, which are polymerized by an enzyme PHA synthase employing intermediates called R-3-hydroxyacyl-CoA. PHAs are thought to be particularly well suited for consumer goods including bottles, films, and textiles because of their impermeability to water and air. Due to the amorphous, soft, and sticky nature of PHAs, which are composed of high molecular weight monomers, also known as medium chain length PHA; mclPHA), polymers must find extensive uses.

Plant cytosols include beta-ketothiolase, the first enzyme in the PHB process. The redirection of acetyl-CoA and acetoacetyl-CoA away from the endogenous flavonoid and isoprenoid pathways is one of the harmful impacts of PHB synthesis in the cytosol. Small alterations in these pathways, which are involved in the manufacturing of a variety of plant hormones and sterols, may have significant impacts on plant development (Poirier et al. 1992). The primary enzyme responsible for creating acetyl-CoA in the cytosol is similar to the phenotypes seen in transgenic plants that produce PHB in their cytoplasm. The plastids are an ideal setting for PHB because they have a strong metabolic flux of acetyl-CoA for fatty acid synthesis and can collect a sizable amount of starch.

The optimum polymer for PHA could not be PHB, which only contains C4 monomers. A more flexible material is produced by adding 3-hydroxyvalerate or longer 3-hydroxy fatty acids, which lower crystallinity. Using a threonine deaminase, propionyl-CoA was introduced to create P(HB-co-HV) co-polymers. This enzyme produces 2-ketobutyrate, which the endogenous pyruvate dehydrogenase complex can convert to propionyl-CoA. P(HB-co-HV) accumulation was caused by the three PHB biosynthetic proteins' coexpression in the plastid (van Beilen and Poirier 2008) (Fig. 2).

A bacterial enzyme called chorismate pyruvate lyase may convert chorismate, which is present in the plastid, to pHBA (CPL). By some unidentified process, pHBA moves from the nucleus to the cytoplasm, at this time it is glycosylated by endogenous uridine diphosphate glycosyl transferases (UDP-GT) and then deposited in the vacuoles. The alternate process involves the conversion of 4-coumaryl-CoA to 4-hydroxybenzaldehyde via a 4-hydroxy-cinnamoyl-CoA hydratase/lyase (HCHL) intermediate. A plant enzyme that has not been discovered oxidizes the bulk of the 4-hydroxybenzaldehyde to pHBA (Fig. 3).

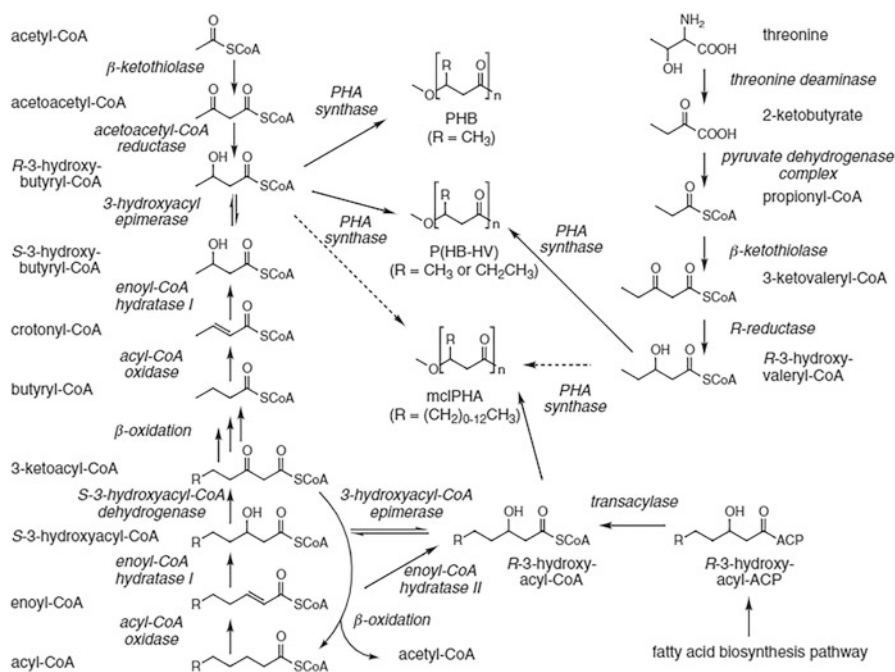


Fig. 2 Pathway for the synthesis of PHA Pathway for the synthesis of 4-hydroxybenzoate (pHBA)

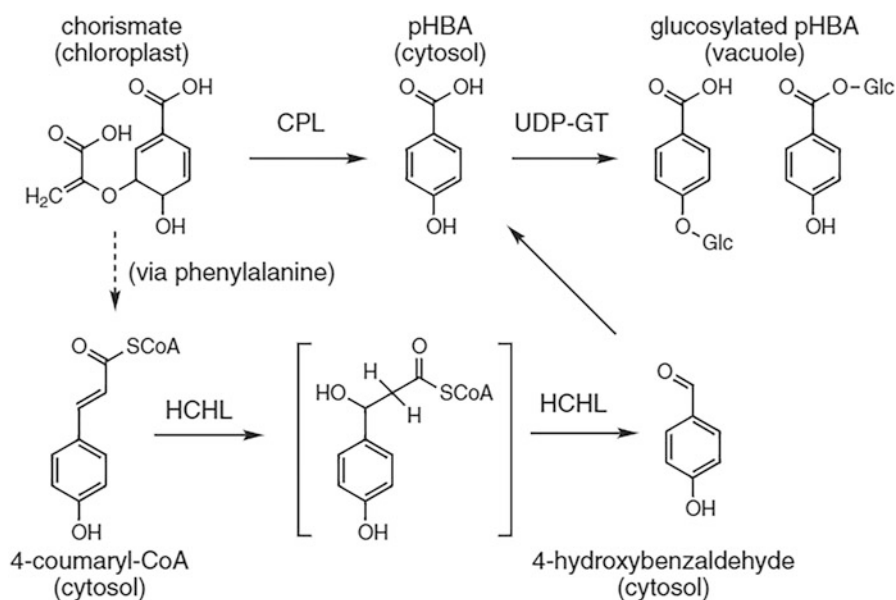


Fig. 3 Pathway for the synthesis of 4-hydroxybenzoate (pHBA)

Chemical Synthesis Produced Polymer (PLA)

The most common example of a biopolymer made from a renewable resource is PLA, a plastic made from maize and starch esters. Recent advances in fiber development and composite engineering provide opportunities for novel resources creations. From recent advanced technology in fiber development and in composite engineering that provide opportunity for novel creations from resources.

It is possible to create PLA by fermenting simple sugars from plant such corns, potato, and sugarcane. Production of PLA is mostly accomplished by enzymatic hydrolysis, fermentation, and catalytic depolymerization (Munoz-Bonilla et al. 2019). In addition to other industrial areas including the agriculture and food packaging industries, the biomedical industry also makes extensive use of PLA.

Produced by Microorganisms

Recombinant DNA or rDNA techniques help to create the chains of polymer, which is almost continuous in length, structure, and orientation.

Polyhydroxyalkanoates

Managing plastic waste has become a serious issue for the environment on a worldwide scale, yet polyester is currently employed in many kinds of applications such as fibers and films. An ecologically friendly polymeric substance, polyhydroxyalkanoate (PHA), may be used to create films and fibers produced by many microbes from renewable carbon resources. PHA may be used to make resorbable medical devices such as meshes, implants, sutures, and scaffolds for tissue engineering. Numerous bacteria can collect these polymers as a carbon source when there is an excess of carbon and nutritional imbalances such as nitrogen, phosphorus, or oxygen constraint and energy storage material. More than 300 species of Gram-positive and Gram-negative bacteria as well as a wide range of archaea can be composed of more than 100 types of different monomers which can act as a carbon or energy storing place (Popa et al. 2014).

PHAs can be made from renewable raw materials and degradation enable for the ecosystem's carbon dioxide and organic compound recycling in the ecosystem by microorganisms, which can act as a climate change buffer. An extracellular PHA depolymerase enzyme secreted by microorganisms causes biodegradation of PHA material. New studies are now being conducted to create PHAs using transgenic plants. The most well-known polymers in the family of polyhydroxyalkanoates are polyhydroxybutyrate (PHB) and poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV). PHAs may be isolated and extruded into hard and flexible plastic films with good moisture and oxygen barrier qualities since they naturally accumulate in microbial populations as their source of carbon and energy. PHAs are able to effectively replace the conventional polymers like polyethylene terephthalate (PET). Most studied among PHAs, polyhydroxy butyrate (PHB) homopolymer and copolyester of β -hydroxy valerate (PHB-co-HV) are used (Popa et al. 2014).

Li and Wilkins (2020) suggest that traditional petroleum plastics be replaced by PHA in the production of a variety of items such as drug delivery system, films, and medical devices. Agro-industrial by-products such as sugarcane, rice straw, peel of orange, and cassava wastewater are the most commonly used low-cost fermentation media for the production of PHA polymer. *Bacillus subtilis*, *Cupriavidus necator*, *Haloferax halophila*, *P. aeruginosa*, and *P. putida* are a few bacterial strains that may manufacture various PHAs from a range of feedstocks (Popa et al. 2014).

A wide range of bacteria accumulate polyhydroxybutyrate (PHB), a polyester from the PHA family, as an internal source of organic carbon and chemical energy. By varying the valerate content, the properties of the PHBV co-polymer can be easily changed. PHB (biodegradable thermoplastic polyester) is considered as analogous to many conventional petroleum-derived plastics. PHB is also biocompatible and may be produced using a sustainable method from both an economic and ecological perspective.

Bacterial Cellulose

Bacterial cellulose (BC) is a naturally occurring nanomaterial that can be renewable and has exceptional mechanical, porosity, water absorption, moldability, and

biological affinity. Still there are vast research and exploration on bacterial cellulose because of the availability. Advanced acoustic diaphragms, the food industry, the medical area, and other fields require low-cost preparation and application, nano-materials mainly focused on their biosynthetic process (Weili et al. 2014; Zhang and Luo 2011). Except with unique three-dimensional porous network structures, the molecular structure of bacterial cellulose and plant cellulose is identical. Compared to plant cellulose, cellulose produced from bacteria is known to have unique properties such as its absence of lignin and hemicellulose, and it is a very pure source of cellulose. Additionally, it possesses a high Young's modulus at both ambient and high temperatures, high degrees of polymerization and crystalline nature (60–70%), a very high water-holding capacity of up to 100 times its own mass, great biodegradability, and a strong biological affinity (Philippini et al. 2020). At the industrial phase, the microbial biopolymers are produced mainly by bacteria and fungi. Several factors can influence the production of biopolymer; apart from the microorganism of choice, optimizing physical factors including pH, temperature, aeration, and fermentation time is necessary to achieve high output yields. It can be found in several forms, including (i) inside the cell, where it serves as a storage area for carbohydrates; (ii) on the cell wall, where it provides structural support; and (iii) as an exopolysaccharide, found in capsules, slime, or biofilm.

The manufacture of bacterial biopolymers such dextran, curdlan, gellan, and xanthan is the principal usage of the agro-industrial biomass. The majority of industrial biopolymer synthesis is accomplished by fermentation of glucose or other simple carbohydrates. The makeup of the media, particularly the sources of carbon and nitrogen, as well as the minerals, additions, and vitamins, must be carefully taken into account in order to achieve maximum production (Philippini et al. 2020).

Xanthan

Xanthan gum (a complex copolymer) is the first commercially viable bacterial polysaccharide generated by bacterial fermentation and is utilized in both food and non-food industries. The bacteria *Xanthomonas campestris* created “repeat units” in the xanthan polymer that have five distinct sugar groups. Gelling agents are used in applications such as cheese spreads, ice creams, puddings, and other desserts whereas industrial applications include pharmaceuticals (being evaluated for sustained drug release), paper manufacturing (used as a modifier), mineral ore processing (used as a biocide), agriculture (acts as plant growth stimulator), and so on (Rao et al. 2014).

Pullulan

Pullulan compounds are formed outside of the cell membrane by numerous species of yeast, mostly by *Aureobasidium pullulans*. They are water-soluble linear

polysaccharide made up of monomers, contain three linked glucose sugars. It has no flavor or smell and is nontoxic. Pullulan compounds exhibit a wide variety of elasticity and solubility qualities, have good heat resistance, and are biodegradable in biologically active conditions. As a result, they can be used as food additives in low-calorie meals and beverages to provide texture and volume in place of starch or other fillers. Since it doesn't degrade in presence of ingested digestive enzymes. Pullulan functions as a plasma expander in the medical field without causing any negative effects. It is entirely eliminated after metabolic conversion. Pullulan also is used in medicinal adhesives and medication carriers. It can be used to solid fertilizers as a binding agent (Rao et al. 2014). Additionally, the potash clays, uranium clays, and ferric hydroxide that are precipitated from slurries used in the beneficiation of mineral ores can be precipitated using this biopolymer.

Glucans

Glucans are homopolymers of glucose, a typical simple sugar found in yeast cell walls. *Saccharomyces cerevisiae* is frequently used as a source of this glucan. About 12–14% of total dry cell mass, glucan is the most prevalent polymer in yeast. Hot alkali treatment makes it simple to separate glucan from yeast cells and eliminate all other cellular components, enabling the recovery of the insoluble glucan substance. Due to its immunomodulatory effects, research has been done on the use of glucan in plants as a powerful antiviral and anti-infective agent. Several studies have shown that glucans can slow the development of tumors. Another characteristic that increases test animals' survival after radiation dosages that would otherwise be fatal is radioprotective. A backbone of glucose residues connected by α -(1,3)-glycosidic bonds makes up glucans, while attached side-chain glucose residues are connected by α -(1,6) connections. Half of the fungal cell wall are made up of the glucan which appear to have therapeutic promise for a number of disorders (Rao et al. 2014).

Applications of Biopolymers

Food Applications

The two main food degradation processes that affect food quality, safety, and shelf life are oxidation and microbial spoilage. Due to their antioxidant and antibacterial properties, biopolymers have a significant impact on food preservation. The possibility of using antibacterial and antioxidant biopolymers as food preservatives leads to an increase in the preference for natural antimicrobial and antioxidative molecules over synthetic ones. This benefits the customer by raising knowledge of the harmful effects of artificial preservatives on health.

Biomedical Application

Biopolymers can be progressively breakdown assimilate and or remove by the body. They are particularly amazing for biomedical technologies including tissue engineering, bone regeneration, dental applications, and controlled drug administration due to the fact that they are made of a variety of harmless components and are thought to be innately biocompatible. Medical device applications employ biopolymers to interface with biological systems. They are also being tested as a matrix for tissue engineering and they can coexist with host reactions in a given use (Park et al. 2008).

Polysaccharides can be used to immobilize enzymes needed for diagnostics or chemically modifying pharmaceutical goods, as well as to encapsulate medications for their progressive distribution (Chaabouni et al. 2013). Exopolysaccharides are used in a variety of industrial processes. One such use is the purification of drugs and enzymes for diagnostic purposes using dextran and its derivatives on a laboratory and industrial scale, which has a direct or indirect connection to medicine. All of these uses make advantage of the functional traits like the ability to form gels and can be employed as vaccinations instead of complete microbial cells or cultures. Thus, undesirable consequences like proteins or lipopolysaccharides are avoided. A number of bacterial EPSs are crucial. As opposed to that due to their intrinsic biological activity as well as their capacity to build polymeric matrices, a number of bacterial EPSs play a significant role in the development of novel medicines. Xanthan, sulfated dextran, and sulfated curdlan for example are antiviral and anticancer drugs. FucoPol is a substance with the potential to be used in anticancer, anti-inflammatory, and immune enhancer medications due to its unique properties, including its high fucose concentration, FucoPol is a substance that has the potential to be employed in anticancer, anti-inflammatory, and immune-enhancer medications. It is rare and challenging to get fucose, which has high demand on the global market. It has been observed that fucose-containing formulations and oligosaccharides have qualities that enhance their usage in medicines (such as anti-inflammatory or anti-carcinogenic substances). EPS can be used as a source which constitute value-added applications (Chaabouni et al. 2013).

Antioxidant and Antimicrobial Applications of Biopolymers

Biopolymers play a vital function in food maintenance due to their antioxidant and antimicrobial properties and rising demand for natural antimicrobial and antioxidative compounds over artificial compounds (Irkin and Esmer 2015). Compounds such as imperative oils, polysaccharides (chitin and chitosan), proteins, and peptides (nisin) are extensively studied and used as antimicrobials, as direct components, or an additive delivered into the food packaging materials. They promise antimicrobial work against various types of food borne pathogens and spoilage microbes. **Natural antimicrobial polymers:** Antimicrobial activity may be added to polymeric materials for a variety of purposes. These include chemically

grafting antimicrobials onto materials, coating antimicrobials on to the polymer surfaces, and integrating antimicrobial compounds directly into polymers. Alginate, chitosan, gelatin, and other bioactive polymers can be employed in the packaging of food goods. Natural biopolymers are superior to manufactured polymers in that they are edible, biodegradable, and renewable (Khan et al. 2014; Rhim and Ng 2007; Shemesh et al. 2015). Some applications about antimicrobial polymers are as follows:

1. Polymeric packaging materials – on polymeric packaging materials, especially for low-density polyethylene (LDPE) films, since LDPE is commonly used as an inner layer in packaging combinations.
2. Biopolymers – Biopolymers may be made from renewable resources such cellulose, alginates, starch, zein from maize, whey protein, aliphatic and aromatic copolymers, and zein from milk. They offer defense against vapor, fumes, and moisture. In addition to these benefits, they degrade more quickly in the environment. Numerous antimicrobial substances, including bacteriocins and plant extracts, were combined to form diverse biopolymers.

Essential oils: The growth of dangerous organisms including *Salmonella* spp., *E. coli*, and *Candida* spp. can be inhibited by essential oils. Oils derived from clove, thyme, and oregano are recognized for their antimicrobial activities, and most active extracts have been oregano and thyme (Iturriaga et al. 2012). Also fennel, coriander, ginger, garlic, pepper, and other spices were suggested (Liu et al. 2017). *Cinnamomum zeylanicum* essential oil is used to examine six *Pseudomonas* species, such as *P. agglomerans*, *P. lundensis*, *P. proteolytica*, and the MIC ranged MIC50 of 3.125 and MIC90 12.50 $\mu\text{L/mL}$.

Modes of actions of essential oils as antimicrobials:

- The cytoplasmic membrane damage
- Coagulation of cytoplasm
- Cell wall damage
- Reduction in intracellular ATP pool

The key step in treating wastewater and sludge recycling is sludge dewatering. Better sludge dewaterability enables reuse and disposal at a lower cost, such as the production of polyhydroxyalkanoates or other bioplastics, the production of building bricks, and the use of sludge as a raw material for the growth of industrial microorganisms (Bhavaniramy et al. 2019).

Environmental Applications

During the wastewater treatment process exopolysaccharides play an important role in sludge dewatering. The key step in treating wastewater and sludge recycling is sludge dewatering. Better sludge dewaterability enables reuse and disposal at a lower

cost, such as the production of polyhydroxyalkanoates or other bioplastics, the production of building bricks, and the use of sludge as a raw material for the growth of industrial microorganisms. Sludge is negatively charged and therefore cationic artificial polymers are used to neutralize the sludge charge, which assists to settle down the sludge. All of this procedures can pollute the surroundings and are costly and consequently security precautions should be observed throughout handling. By using biofloculants/biocoagulants produced by bacterial strains derived from activated sludge help to overcome these problems.

The bioactive compounds microbes during their development mostly consist of carbohydrates, proteins, lipids, DNA, and RNA. Microorganisms are also capable of thriving in a variety of conditions. Microbial EPS reacts with the sludge particles to contribute significantly to the biological flocculation process. The microbial EPS might be non-ionic or include both cationic and anionic charges. The concentration and properties of EPS have a significant impact on its capacity to settle sludge. There is often an ideal EPS concentration for sludge settling. Sludge properties, EPS biochemical features, operational factors in a wastewater treatment plant, and kind of dominant microbial community in that treatment plant all have an impact on the concentration of EPS generated by sludge (Tyagi et al. 2002; Yezza et al. 2004).

Packaging

Polymers in packaging are affected by using their chemical structure, molecular weights, and crystallinity, and foods have more strict packaging requirements than solid commodities; however, all these physical characteristics required in packaging depend equally on the items to be packaged and the settings in which the applications will be kept, and products that will be frozen for an extended period of time require special packaging. The focus of recent research has been on developing polymeric matrices with the best homes (such as clarity, barrier and mechanical characteristics, or biocompatibility) for a number of applications, including coatings and packaging for consumable foods. In bulk packaging, bioplastics provide a variety of additional advantages over traditional plastics, including the same process that is utilized for conventional materials may be used to make bulk packages out of biologically produced polymers. These results show that they are just as significant as traditional plastics in terms of physical, thermal, mechanical, and barrier qualities (Yasuhiro and Yoshimitsu 2010). It plays a crucial part in preserving food quality, shielding the packaged product from the elements, and enhancing the qualities of the food during storage. However, often used packaging made of synthetic, non-biodegradable polymers seriously pollutes the environment. Bioplastics (such PHBV and PHB) can break down in compost, soil, streams, lakes, seas, aerobic and anaerobic sewage sludge, and other aspects of nature. Compost made in section from bioplastics improves soil natural content, water and nutrient retention, and suppresses plant diseases while lowering chemical inputs. Furthermore, it has been

demonstrated that starch-based bioplastics disintegrate 10–20 times faster than conventional plastics.

Burning conventional plastics releases toxic vapors that can be harmful to the environment and human health. If any bio-based films are burned, just a little amount of hazardous chemicals or gases are discharged into the atmosphere. In phrases of safety, the degradation research observed that greater than 90% of samples degraded in 10 months, based on weight loss and CO₂ production measurements.

Utility of a different bio-composite in process of making the laundry baggage for medical institution and different institutions, where the bag dissolves at some point of the washing and biodegrades after disposal into sewage. Solubility of water-soluble biocomposites is dependent on the quantity, molecular weight, and crystallinity. In anaerobic sewage sludge, for example, PHBV mineralizes to CO₂, water, and a little amount of methane over the course of 30 days (Chaabouni et al. 2013).

When comparing samples of bioplastic compost made by combining the test material with organic waste to samples of reference compost made simply with organic waste, it was discovered that when tested on plants, it did not emit compounds hazardous to the plants or the environment. Bio-based polymers contain more diversified stereochemistry and side chain architecture than traditional plastics generated from petroleum, giving research scientists additional options for customizing the qualities of the final packaging material. With these added benefits and nearly identical qualities to PVC, Nylon, PP, bioplastics can be used in the huge packaging to replace petroleum-based plastics that pollute the environment due to their non-degradability and toxic gas emissions.

Edible Coating with Silver Nanoparticles: On a variety of meals, including fruit and vegetables, meat, and cheese, the practical application of edible coating with integrated silver nanoparticles has previously been tried. Numerous research have examined the application of coating materials on fresh, little processed fruit and vegetables as a means of reducing spoiling and lengthening the shelf life during post-storage. All of these investigations demonstrate that silver nanoparticles, which were used as active agents and inserted into the polymer matrix, demonstrated wide antibacterial action, primarily against microorganisms that are liable for lowering the microbiological quality of food items. The advantages of adopting active packaging like as films and coatings relate to the slow and progressive release of antibacterial drugs onto the food surface from the polymer matrix, where the bulk of contaminating by spoilage or pathogenic bacteria occurs for most goods. The quantity of preservatives often injected directly into the main body of food might likewise be minimized or significantly reduced thanks to this use.

Food packaging systems with natural antimicrobial agents: Directly inserting incorporation of antimicrobial chemicals into packaging films, their coating on packaging films, and the use of polymers in packaging materials can all result in an antimicrobial packaging system. Antimicrobial packaging increases the shelf life, security, and quality of many foods due to its exceptional ability to prevent growth of microorganisms in non-sterile foods and reduce the danger of post-contamination of sterilized goods.

Classification	Antimicrobial agents and biopolymers
Polysaccharides and derivatives	Starch, chitosan, pullulan, natural gums
Proteins, enzymes, bacteriocins	Corn-zein, wheat-gluten, peanut, milk, collagen/gelatin Nisin, soy-protein isolate, whey-protein isolates
Chelating agents	EDTA

*Essential Oils significant antibacterial activity have been observed for extracts of cinnamon, oregano, and thyme against a variety of pathogens, including *Listeria monocytogenes*, *E. coli*, *B. thermosphacta*, and *P. fluorescens*. EOs considerably travel through the lipids of bacterial cell membranes, disturb the architecture of the cell walls, and make them less permeable because of their hydrophobic nature. For instance, trans-cinnamaldehyde, one of main EO components, has the ability to inhibit the development of *S. typhimurium* and *E. coli* by lowering intracellular ATP levels. The periplasm and deeper parts of the cell are also accessible.

Agricultural and Agro-Industrial Applications

Nowadays, controlled release of insecticides and nutrients, soil fertilization, seed coatings, and crop protection all include the usage of polymers like plastics, coatings, elastomers, fibers, and water-soluble polymers. Biodegradability plant pots, erosion control netting, are just a number of the agricultural uses for bioplastics. Bioplastics are also being investigated as crop mulching materials in fields and as agricultural plant containers. Composting has a one of a kind property that can significantly improve the state of the soil. Along with degradable plant pots, stakes, erosion control netting, and mulch film, they also serve a variety of other purposes. Except in political geographies where nondegradable plastic items are prohibited, receiving a top class for low-value merchandise such as disposable ware or horticulture functions will be pretty exceptional (McCormick and Lichatowich 1979).

Because of their non-Newtonian behavior and high viscosity in aquatic conditions, EPSs produced by bacteria are frequently used in high-value applications. These applications include thickening, stabilizing, binding, and structure building. They should be able to retain their abilities when incorporated into components with significant pH and ionic strength fluctuations, as well as under the influence of additional food ingredients, when utilized in food products. One of the materials found in agricultural wastes most frequently is biopolymers. Recovery of biopolymers found in trash is typically viewed as a backup option for agro-industrial activities. A lot of the time, agricultural and in order to produce energy, fermented chemicals, composts, and/or biofuels, industrial wastes are burnt. Although each hexose monosaccharide, which is only composed of C, O, and H in a biopolymer main chain, has a primary bond energy of C, O, and H, the energy losses of converting a biopolymer into fuels are quite substantial. Caseins, collagen and gelatin, chitin and chitosan, as well as alginates, bacterial cellulose, poly-hydroxyalkanoates, β -glucans, and xanthan can be obtained from animal feces.

Conclusion

A brand-new class of materials that is still in development, biopolymers are used to make things like food, clothes, medical supplies, packaging, food additives, engineering plastics, water treatment, and other necessities of everyday life. Biodegradable biopolymers can be made from natural-origin monomers as well as those that are derived from animals, plants, microorganisms, and chemicals. Even while biopolymers are found throughout nature, only a few numbers of plants and animals are heavily utilized for their commercial manufacture.

Non-biodegradable plastics are being used more and more in both terrestrial and aquatic ecosystems, which has a negative impact on the environment. Because of their biodegradability and environmentally friendly production processes, biopolymers are valuable replacements for non-sustainable goods. They also have a broad range of uses, including in the food industry, agriculture and agro-industry, packaging, biomedicine, and other fields. The creation of biodegradable polymers to replace their equivalents made of fossil fuels that are not biodegradable has been the focus of recent investigations for the creation of bulk biopolymers. These replacements for polyolefins include polyhydroxyalkanoates and polylactic acid. To fulfill the demands of customers for more ecologically friendly packaging for more natural products, bio-based films, or biopolymers, it's critical to improve the quality of numerous items. This strategy will continue to be crucial in the food business. Additionally, biomass may be used in a two-step procedure to create biobased polymers, where chemical modification of natural polymers can be used to create sustainable polymers from renewable resources.

Due to the accessibility of genetic engineering techniques and metabolic pathway engineering methods, which open up a constantly expanding design space for the creation of biopolymers with desirable material characteristics, bacteria continue to be suitable polymer production organisms. Additionally, if the relevant biopolymer is a key component of virulence, additional understanding of the molecular processes behind biopolymer production might be exploited to create customized biosynthetic enzyme inhibitors. There is, however, relatively little utilization of bacteria to make biopolymers, namely polysaccharides. In the entire hydrocolloid market, polysaccharides derived from plant, algal, and animal sources continue to dominate. This is mostly due to increasing pricing for bacterial polysaccharides, which are a result of the high cost of frequently utilized carbon sources and related downstream expenses.

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Recent Approaches Toward Bacterial Nanocellulose (BNC) Synthesis

3

Chhavi Sharma  and Puneet Pathak 

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C. Sharma (✉) · P. Pathak

Avantha Centre for Industrial Research and Development (ACIRD), Thapar Institute of Engineering and Technology Campus, Patiala, India

e-mail: chhavisharma19@gmail.com; puneet@avantharesearch.org;

pathakpuneet26aug@gmail.com

Abstract

Bacterial nanocellulose (BNC) is a natural material of immense industrial applications and has relevance in multipurpose areas. BNC has ascended as a natural polymer of substantial status in various technoindustrial applications owing to its implausible biological and physicochemical features. It has potential in the bio-medical, food, paper, textile, and cosmetics industries. The feasible utilization of BNC in multidimensional areas is widely reliant on its production. However, the production cost, paucity of scale-up techniques, and the high prices of BNC-based products are the crucial facets that are the roadblocks to the commercialization of BNC. Therefore, the additional impetus is mandatory for making this green material a competing product while simultaneously being cost-effective. Despite the extensive research work in the field of BNC production, the aim of cost-effective large-scale BNC production is still far from reality. Thus, constant efforts are obligatory for viably obtaining BNC by cutting down the synthesis price, yield enhancement strategies, and overall functionalization to improve the physical, mechanical, and biological characteristics. In this chapter, we attempted to summarize recent reports on the BNC production technologies and the advancement in BNC synthesis strategies, such as static intermittent fed-batch technology, cell-free extract technology, and bioreactor-assisted BNC production. It also reflects various approaches toward significant BNC synthesis by addressing the challenges in high-quality BNC production. The scale-up strategies, like the use of novel substrates and strain improvement, have been conferred in detail.

Keywords

Bacterial nanocellulose · Multipurpose biomaterial · Sustainable production technologies · Static intermittent fed-batch technique · Cell-free extract method · Bioreactor-assisted technologies · BNC scale-up strategies · Production challenges

Introduction

Bacterial nanocellulose (BNC) has ascended as a biomaterial that has grasped the attention of researchers at both the industrial and academic levels in the last decade. It has emerged as a biopolymer of imperative grade owing to its incredible biological and physicochemical features like high tensile strength, crystallinity, biocompatibility, thermal stability, and hydrophilicity. BNC materials have received the status of generally recognized as safe (GRAS) and thus suitable for wide-ranging purposes. BNC has gained the designation of “biocellulose” credited to its dynamic properties and potential. The exclusive properties of BNC made it suitable for various techno-industrial applications. Its most prodigious application exists in the biomedical area. It also has conspicuous potential in the biomedical field, pharmaceutical industry, food industry electroconductive composites, wastewater treatment, packaging industry, pulp, and paper industry, textiles, and even the construction field (Jozala et al. 2016). Despite the mounting status of BNC in versatile technoindustrial

applications, the production process still encounters several challenges which include long propagation time, low yield with limited cellulose layer thickness, and high cost of production. All these factors generate considerable barriers to large-scale BNC production and subsequently its utilization for varied applications (Sharma and Bhardwaj 2019a).

The increasing appliance of BNC and BNC-derived products in several branches of industry gave rise to intensive studies on the improvement of its synthesis efficacy while endeavoring toward lowering the costs of synthesis of BNC. The studies directed to comprehend the fruitful large-scale synthesis of BNC need much investigation.

The leading necessity of time is to focus on new research avenues for prolific industrial-scale BNC synthesis. In the twenty-first century, the main research focus is on the optimization of BNC production parameters using conventional media along with static and agitated strategies (Dubey et al. 2018). The substantial production methods, different aspects, and parameters for high-quality BNC synthesis are still the main focus of the research besides its applications (Campano et al. 2016). A lot of efforts have been taken by different research groups to augment BNC yield by altering the media composition, production protocols, and parameters or strain improvement at the genetic level. The medium accounts for around one-fourth of the overall production cost. Recent research has focused on the use of alternative media with inexpensive nitrogen and carbon sources such as effluents from the paper industry, agro-industrial wastes, textile waste, etc. Diverse substrates like industrial wastewater often have large amounts of sugars such as fermented black tea broth (Sharma and Bhardwaj 2019a), candied jujube-processing industry wastewater (Li et al. 2015), hot water extract of wood (Kiziltas et al. 2015), and agro-industrial wastes like molasses (Tyagi and Suresh 2016), therefore pineapple waste and coconut water (Vazquez et al. 2013; Jagannath et al. 2008) instead of conventional sugar sources are gaining importance. An alternative approach for upsurging BNC synthesis is by adjusting the synthesis parameters, which involved investigating a diversity of bioreactors to search for the appropriate synthesis environments for the particular bacterial strain, both in a static or agitated mode. An assortment of methods, ranging from batch to fed-batch bioreactors-assisted techniques, as well as several specific devices, are available for the synthesis of BNC (Watanabe et al. 1998; Bae et al. 2004). Numerous comparative research works had been done to investigate a better method of BNC synthesis by utilizing static and agitated production modes (Czaja and Romanovicz 2004; Zywicka et al. 2015). The physico-chemical, mechanical characteristics and supramolecular structure of BNC are strictly driven by its cultivation strategy, therefore the BNC production method depends on its usage. Another option to attain considerable yield is the selection of a high-efficiency bacterial strain. Meanwhile, new BNC-producing strains are been emanated (Castro et al. 2012).

Therefore, the cost-effective BNC synthesis with new substrates along with upgrading the synthesis by amended bioprocess strategies is mandatory to achieve entirely commercialized BNC for various applications in the industry, particularly for bio-based purposes. Currently, altered strategies including cell-free system, static intermittent fed-batch technique, and advanced reactor-assisted bioprocesses like

advanced rotary disc bioreactor systems, etc. have extensively attained significance as these technologies can enhance BNC synthesis.

The realization of abundant industrial-scale BNC synthesis is a prerequisite for exploring its comprehensive potential in different technological applications. Thus, this chapter provides information about the existing status of BNC, describing synthesis technologies and various types of substrates applied until now. It emphasizes the approaches for intensifying BNC yield and methods to scale up the BNC productivity toward commercialization by overcoming the roadblocks. Therefore, this chapter will certainly unravel the diverse hitches allied with prolific large-scale BNC production. The current scenario in BNC production and typically used static intermittent fed-batch technology, cell-free extract technology, bioreactor-assisted technology, and future perspectives of the BNC production strategies are presented and reviewed.

Importance of Bacterial Nanocellulose over Other Nanocellulose Types

The three classes of nanocellulose are: (1) cellulose nanocrystals (CNC)/cellulose nano-whiskers; (2) cellulose nanofibrils (CNF); and (3) bacterial nanocellulose (BNC), i.e., often called microbial cellulose (Gama et al. 2016). CNC and CNF are mainly extracted from various sources such as woody material, cotton fibers, flax and hemp fibers, potato tuber, wheat straw, sugarcane bagasse, algae, etc. (Gama et al. 2016).

If plants are abundant sources of cellulose, what is the need for bacterial cellulose? This question is quite germane when we talk about the importance of bacterial nanocellulose. The answer exists in the fact that most of the plant-based cellulose comes from the harvesting of a wide range of natural forests. Harvesting of tropical equatorial forests is the major cause of the destruction of the ecosystem and its associated species, land erosion, and pollution. Contrary to CNC and CNF, BNC is naturally synthesized mainly by the bacteria *Acetobacter xylinum* in pure cellulosic form and does not involve various processing steps to eliminate unsolicited impurities or contaminants like lignin, hemicellulose, and pectin (Fig. 1). However, extraction of CNC and CNF is generally accompanied by de-structuring strategies using chemical treatments such as acid-based hydrolysis, kraft pulping, bleaching, etc., which make their extraction process prolonged and laborious. Such chemical extraction makes CNC and CNF a lot unsuitable for biomedical use and also presents an environmental concern.

Thus, there is a logical requirement to quest for alternative sources of cellulose. Bacterial nanocellulose has some discrete features unlike the cellulose obtained from other sources. Firstly, BNC is generated as a never-dried film; secondly, unlike plant-based cellulose, the absence of lignin and hemicellulose components in BNC avoids the use of hazardous chemical treatment required during its synthesis; and thirdly, BNC has great mechanical strength and tailorable physical, chemical, and biological properties.

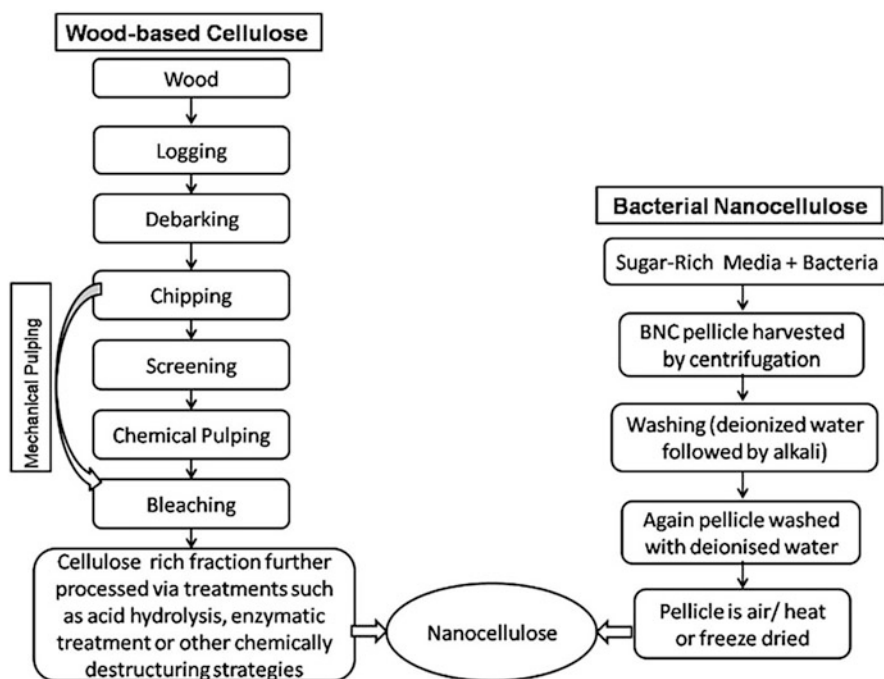


Fig. 1 Comparison of wood-based nanocellulose and bacterial nanocellulose synthesis process

Furthermore, the versatile application of BNC (Fig. 2) itself dictates the splendid worth of BNC. Applications of BNC in the biomedical field include scaffold fabrication; fabrication technologies for innovative structures and medical devices; tissue engineering functional tissues; regenerative medicine for in vivo replacement of functional tissues and organs; nanoparticulate systems for delivery of therapeutic agents and vaccines; and biocompatibility and toxicology studies. Other applications are the production of edible, eco-friendly packaging films and coating for the food industry; production of superior quality paper; development of nanocellulose-implanted air and water purifying systems; development of green cosmetics; fabrication of eco-friendly, surface-modified smart fabrics from microbial cellulose; nanocellulose-based sustainable dyeing strategy, etc.

Bacterial Nanocellulose (BNC): Biosynthesis Pathway and Its Properties

A. J. Brown in 1886 revealed the development of viscous gel-like growth casing the upper surface during the fermentation of vinegar (Brown 1886). The responsible organism for this gelatinous mass was identified as *Gluconacetobacter xylinus* (*G. xylinus*) and the resultant mass was identified as bacterial nanocellulose. In

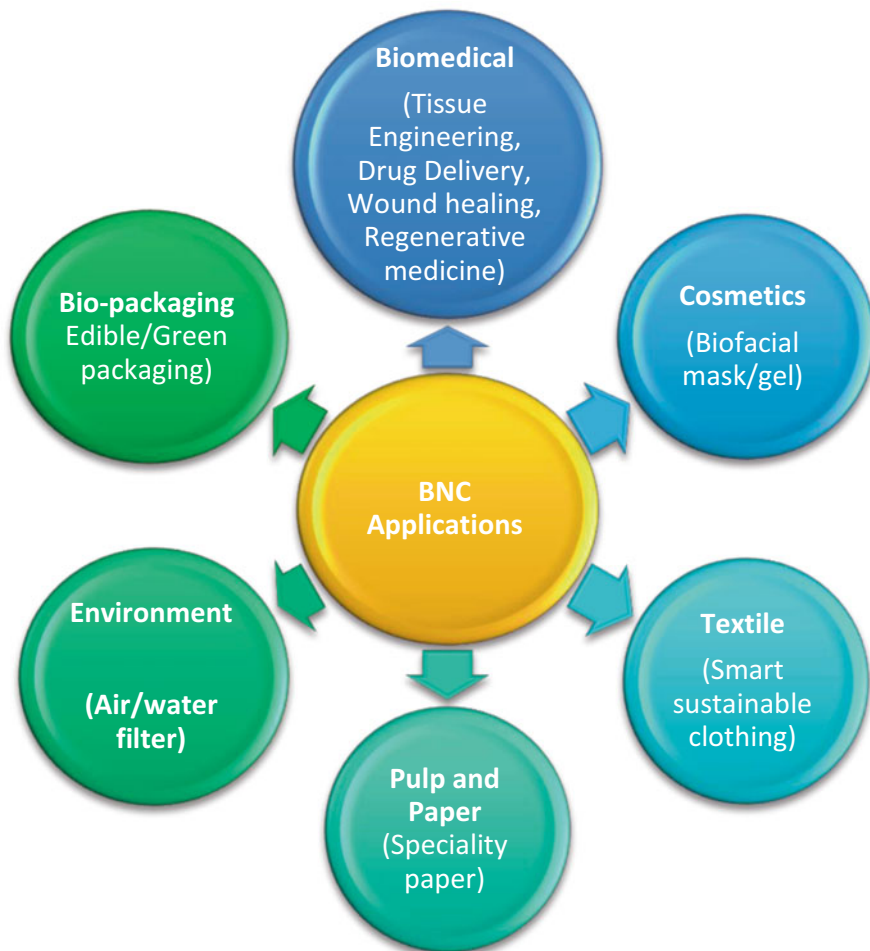


Fig. 2 Multidimensional uses of bacterial nanocellulose

1954, Hestrin and Schramm started evaluating the elementary metabolic processes of *G. xylinus* and developed a typical medium for its culturing to obtain an optimized BNC production at a laboratory scale. They have used carbon source as D-glucose and yeast extract as nitrogen and vitamin sources. These two discoveries are the leading fundamentals for designing all the available BNC production methods. Bacterial genera that are identified as nanocellulose synthesizers include *Acetobacter*, *Bacillus*, *Achromobacter*, *Sarcina*, *Agrobacterium*, *Escherichia*, *Rhizobium*, *Azotobacter*, *Enterobacter*, *Aerobacter*, *Klebsiella*, and *Salmonella*. BNC is produced by all these bacterial strains as a guarding structure against harsh chemical

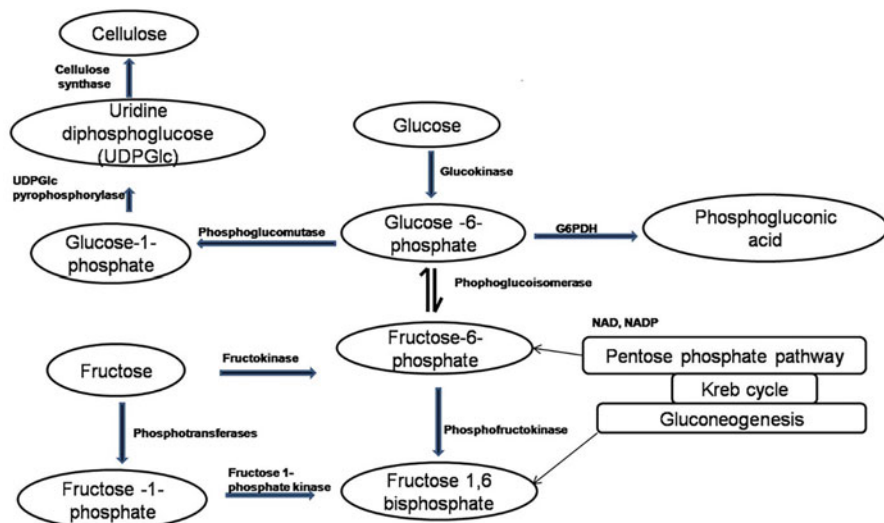


Fig. 3 Biochemical route of BNC synthesis by *A. xylinum*. *NAD* nicotinamide adenine dinucleotide, *NADP* nicotinamide adenine dinucleotide phosphate, *G6PDH* glucose 6-phosphate dehydrogenase

milieus and ultraviolet radiation. Nevertheless, *Gluconacetobacter xylinus*, a gram-negative bacterium, is the utmost predominant BNC synthesizer. It was earlier recognized as *Acetobacter xylinum* (Mikkelsen et al. 2009). Bacterial cellulose synthesis occurs through a bottom-up approach that generally involves four stages: (a) monosaccharides stimulation via the development of sugar nucleotides; (2) assemblage of repeating units of sugars by polymerization; (3) within-time accumulation of acyl units (if existing); and (4) exudation of BNC from the cytoplasm of the cell to the exterior via plasma membrane (Fig. 3) (Siro and Plackett 2010; Benziman et al. 1980). X-ray diffraction studies reveal the similarity between the chemical structure of cellulose derived from *Gluconacetobacter xylinus* and plants. Its molecular formula is $(C_6H_{10}O_5)_n$ with repetitive subunits of $\beta(1,4)$ D-glucose. At the plasma membrane level, the enzyme cellulose synthase is responsible for synthesizing BNC. Roughly 200,000 glucose molecules can be polymerized each second by *G. xylinus* into β -1,4-glucan chains, thus discharging a thread-like packet of warped microfibrils into the nearby milieu (Ring 1982). These ribbons relate to the cellular division progression and persist elongated sideways with the cell envelope (Ring 1982). In a static culture of *A. xylinum*, these ribbon-like fibrils gather to produce a mat-like surface at the interface of air medium which is called a pellicle. The bacterium grows by consuming a range of substrates and excretes nanocellulose. Hence *A. xylinum* is used as a classic bacterium for BNC synthesis because this can produce nanocellulose as an extracellular deposit and is free of impurities. Moreover, the nanocellulose formed is metabolically inert in comparison to plant-based cellulose (Reiniati et al. 2017).

BNC is a biomaterial of outstanding worth in vital research as well as industrial applications. This is because of its intrinsic nanostructure and multidimensional properties like appropriate mechanical strength, hydrophilicity, etc. (Table 1). Figure 4 shows the nanostructure morphology of BNC fiber via field emission

Table 1 BNC properties: conferring about its substantial applications. (Adapted from Sharma and Bhardwaj 2019a)

S no:	Properties	Applications
1.	Resistance to fiber lift and absorbance	Paper-like wound dressings
2.	Strength in the wet state: the capability to resist acute temperature	Adhesive labeling on clinical specimens like blood
3.	Inert nature	Packaging of surgical instruments and medicines
4.	Endure tremendously high temperature	Easy disinfection by gamma radiations or steam required for packaging of sterile equipment
5.	Biocompatibility: no cytotoxicity	Fabrication of nanocellulose-incorporated implants, wound dressings, cellulose-based diapers, and facial masks
6.	Biodegradability	Scaffold fabrication
7.	Mechanical strength	Load-bearing polymer for various applications, such as dental and bone tissue engineering implants
8.	Application-based tailorable nature	Enrichment in obstruction property, tensile strength, oil permeability, air permeability, and antimicrobial properties can be attained by BNC functionalization for varied usage

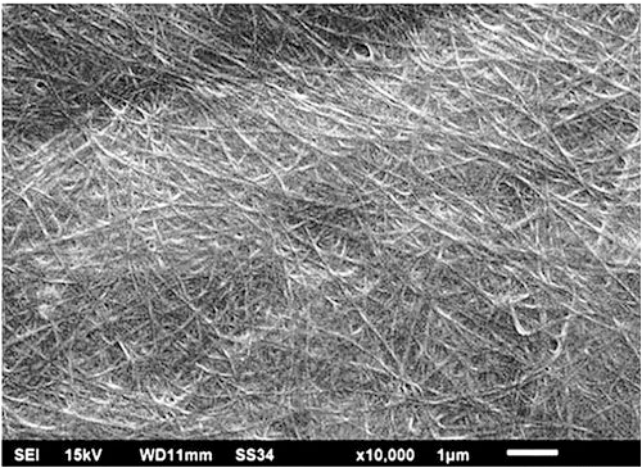


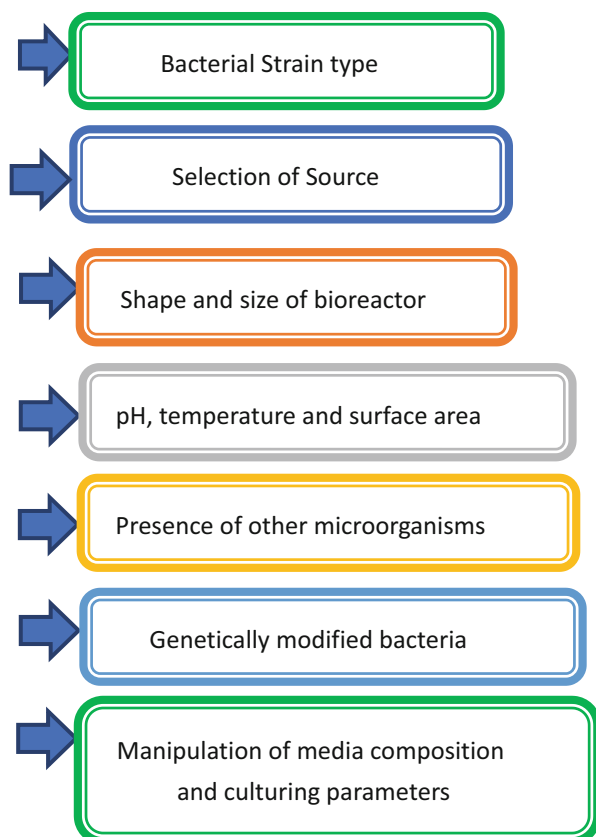
Fig. 4 Morphology of BNC by FE-SEM. (Adapted from Sharma et al. 2021b)

scanning electron microscopy (FESEM). Depending upon the synthesis mode and cultural parameters, BNC morphology can differ.

Factors Influencing BNC Production

Independent of the synthesis technique or the strain used, the process of BNC production could likewise be depicted by a great number of additional factors, together with the constituents of the media, its pH value, oxygen tension, and temperature (Chao et al. 2000; Jonas and Farah 1998; Krystynowicz et al. 2002). Several additives, such as organic or inorganic substances, water-soluble polymers (Yoshinaga et al. 1997), and nanoparticles of metal oxide and metal (Serafica et al. 2002) also affect BNC productivity. Figure 5 depicts some of the factors affecting BNC synthesis. In all cases, the optimization of the synthesis conditions shows an explicit part during the scale-up from investigational (grams/day) to trial (kilograms/

Fig. 5 Factors affecting bacterial cellulose formation



day) or manufacturing scale (tons/day). Furthermore, other parameters and tactics that must be considered and adapted include decontamination of the apparatus, sturdiness, and firmness against corrosion of the reactor vessel components, uniformly dispersed supply of medium and air, defense against impurities, and biological conditions. Though an assortment of methods for BNC synthesis in numerous styles has been anticipated in the last 20 years, in the manufacturing process, BNC is virtually solely synthesized statically in shallow containers utilizing negligibly automated methods. Consequently, the comprehensive commercialization of BNC has been struck by the dearth of competent systems and voluminous synthesizing price of high-quality BNC (Gama et al. 2016).

BNC Production Technologies: Current Status

BNC generation can occur through several techniques ranging from the conventional static synthesis like batch and fed-batch to constant synthesis techniques such as bioreactors and some specialized devices-based synthesis. The choice of production technology is highly influenced by the end use of BNC. Furthermore, the mechanical, physical properties and supramolecular structure of BNC are quite dependent on the method of BNC production (Gama et al. 2016).

To outstretched the array of commercial BNC applications, access to numerous forms of BNC with tailorable supramolecular structure and various material properties became a requisite. Thus, for the potential scaling-up of future commercial production processes, diverse forms of synthesis mode (Table 2) moderately mingled with in situ variations by supports or additives have been documented. By varying the method of synthesis and culture conditions, BNC can be obtained in numerous types and sizes, e.g., flat BNC foils or fleeces, spheres, thin pellicles, fibrous aggregates, tubes, or as BNC pulp. Until now, flat BNC materials have attained industrial importance owing to their self-effacing, nontechnical static cultivation in diverse vessels. Static cultivation has remained an industrially feasible method owing to the colossal utilization of BNC fleeces and foils in the biomedical industry (e.g., tissue engineering and regenerative medicine, wound dressing, drug delivery patches, etc.) (Czaja and Romanowicz 2004) and technical appliance as membranes or material for filtering (Sokolnicki et al. 2006). BNC is typically synthesized as flat sheets/films by conventional static synthesis on diverse permeable sheaths (Putra et al. 2008) or solid phases like silicone rubber (Onodera et al. 2002), agar (Brown et al. 1992), etc. The subsequent foils/fleeces classically possess a three-dimensional (3D) network of uniformly surfaced homogeneous nanofibers. The characteristics of the resultant BNC can be tailored by numerous altering parameters. In 1989, Williams and Cannon investigated the variation in thickness of flat BNC by synthesizing it using diverse *Gluconacetobacter* strains. The thickness of the BNC film can also be controlled by changing the culture medium volume, time of cultivation, and type of container used (Borzani and De Souza 1995; Levy et al. 2004). Besides BNC production under static conditions, synthesis under stirring and shaking (Watanabe

Table 2 Concise summarization of available BNC synthesis methods

Production strategy	Summary	Asset	Drawback	References
Static culture	<ul style="list-style-type: none"> • Mixing of media components at the initial stage • Requirement of the tray for production • Production appears at the air-liquid medium interface 	<ul style="list-style-type: none"> • Simplest • No need for complex equipment 	<ul style="list-style-type: none"> • Effortful and tedious • Uncontrolled or unmonitored fermentation condition • Cellulose production in the form of pellicle and reticulated cellulose slurry • Unsuitable for commercial production 	Adebayo-Tayo et al. (2014)
Static intermittent fed-batch	<ul style="list-style-type: none"> • Need for a fixed quantity of freshly prepared media to growing cellulose containing pellicles at periodic intervals 	<ul style="list-style-type: none"> • Simple process • Higher production than standard static culture strategy • Suitable for commercial production 	<ul style="list-style-type: none"> • Unmonitored fermentation condition • Cellulose production in the form of pellicle and reticulated cellulose slurry 	Dubey et al. (2018)
Cell-free extract	Release of essential enzymes directly into the media for BNC production after cell lysis using mechanical/thermal/enzymatic treatments	<ul style="list-style-type: none"> • Simple process • Suitable for commercial production in a short time • Higher yield 	<ul style="list-style-type: none"> • Uncontrolled or unmonitored fermentation condition 	Ullah et al. (2015)
Agitated culture	<ul style="list-style-type: none"> • Agitation at around 90–100 rpm • The rapid growth of cells due to agitation 	<ul style="list-style-type: none"> • Applicable for commercial production • Overcome the limitations, such as – diffusion, controllability, and scale-up, faced during static culture strategy 	<ul style="list-style-type: none"> • No pellicle formation. • Production of irregular shaped sphere-like cellulose particle • Possibility of culture mutation and culture instability due to agitation resulting in low productivity and inability to produce cellulose, respectively 	Hu et al. (2013) and Tanskul et al. (2013)

(continued)

Table 2 (continued)

Production strategy	Summary	Asset	Drawback	References
Bioreactors: rotary disc bioreactor (RDBR), advanced RDBR, and airlift reactor	<ul style="list-style-type: none"> • Alternate based on the theme of rotating biological contactor (RBC) • Use of rotating discs for soaking the microorganisms in liquid media after half rotation of the disc and then expose them to air in the other half of rotation 	<ul style="list-style-type: none"> • Higher productivity • Easy to handle • Easy to scale-up 	<ul style="list-style-type: none"> • No major drawback, if operating under controlled conditions and using suitable medium 	Krystynowicz et al. (2002), Pa'e (2009), and Sharma et al. (2022)

and Yamanaka 1995) and some altered measures (Table 2) employing these methods (Chao et al. 2001; Wu et al. 2001) are vital for exemplary BNC production.

Static Method

Since the advent of exhaustive research on BNC in the mid-1990s, static synthesis has attained the status of a classic BNC synthesis method. This type of synthesis is still observed as the ideal manufacturing technique to produce BNC in flat form with outstanding and reproducible structures and properties for market applications. The dessert “nata de coco,” is soundly recognized as a primary existing BNC-based edible foodstuff in the food industry of Asia. It is generally statically produced in the form of sheets. In one of the studies, BNC synthesis was enhanced by occasionally providing culture media from underneath the synthesized BNC pellicles (Toda et al. 1994).

Benefits and Limitations of Static Synthesis Method

As BNC was formed at the air-liquid interface, therefore, static synthesis requires a large production zone for producing a reasonable amount of BNC. Alike the agitated method in deep tank fermenters, the static method is not at all automated and hence needs additional labor-intensive work and higher worker expenses. Besides this drawback, in the static mode, the change in the nutrients amounts during fermentation restrained the required synthesis time and increase the demand for regular costly holding periods.

Agitated Method

BNC production of various types such as irregular masses, fibrous suspension, pellets, or spheres can be attained by agitation (Krystynowicz et al. 2002). The synthesis of defined sizes BNC with tailorable surface characteristics can be obtained by using certain cultivation parameters. Studies showed large-scale industrial production of BNC by agitation method (Czaja and Romanovicz 2004). Many studies present a view that static cultivation affords only a constrained amount of BNC yields escorted by synchronized use of large surfaces and space demands. Unlike in static synthesis, the production limit of BNC can be surpassed by utilizing agitated synthesis in fermenters leading to feasible BNC production. This is because shear stress during agitation could lead to disruption of bacterial cells, resulting in low productivity. This often causes spontaneous mutation of bacteria from active cellulose producers to non-cellulose-producing strains. The agitated methods hold their peculiar inimitable potential for future industrial usage. The consequential BNC spheres, filaments, or pulps are suitable for wide end use, for example, as an additive for papermaking and fiber reinforcement of synthetic or biopolymers, etc.

Benefits and Limits of Agitated Cultivation Forms

When the agitation technique is used in stirred aerated fermenters or submerged cultures in a shaken flask, the synthesis duration can be ten times decreased in contrast to static fermentation (Gama et al. 2016). Nevertheless, the concept of augmenting the BNC productivity by increasing the air drift rates was a failure as it led to a reduction in the BNC productivity. Limited *Gluconacetobacter* strains, such as ATCC 53582, are suitable for agitated cultivation. This is because shear stress during agitation could lead to disruption of bacterial cells, resulting in low productivity (Hestrin and Schramm 1954; Krystynowicz et al. 2002). This often causes spontaneous mutation of bacteria from active cellulose producers to non-cellulose-producing strains. A study by Park et al. (2003) reported the alteration from cellulose-synthesizing to cellulose-nonsynthesizing *Gluconacetobacter hansenii* PJK strain due to shear forces and subsequent cessation of BNC production.

Recent Advancements in BNC Production Technologies

Presently, the research direction focuses on enhancing BNC productivity, either by altering the medium composition, production methodology, and conditions, or strain improvement of BNC producers. Over 30% of the BNC production cost depends on the type of medium used. Thus, one of the directions of research is to focus on experiments with inexpensive nitrogen and carbon sources like food leftovers, wastes from the agriculture and textile industry, paper industry effluents, etc.

Optimization of the production parameters by investigating various types of bioreactor-assisted approaches along with different strains and culture medium is another research strategy to get access to the appropriate synthesis conditions for particular bacteria under static or agitated mode. The selection of a high-yielding

strain for BNC production is a crucial step in the mass production of BNC. Some of the BNC synthesis techniques that differ from the conventional static and agitated methods are briefed below.

Semicontinuous Generation of Flat BNC

One of the effectual methods is the semicontinuous synthesis of BNC devised by Kralisch et al. (2010). In this method, BNC was synthesized as foils and fleeces in plan form. He utilized a horizontal lift reactor (HoLiR) idea for generating unremitting sheets of BNC with tailorable thickness and desired dimension. The planar BNC material so formed has a 3D network morphology. This valuable union of constant dispensation and virtually static cultivation resulted in the uniform synthesis of the anticipated size BNC. It was raised and carried out of the reactor constantly utilizing a separator device placed at the reverse end of the reactor. The extraction step was performed without disturbing the medium surface to defend the BNC sheet under synthesis.

An evenly surfaced plane BNC sheet having a thickness of 0.5–1.5 mm/day was obtained after the prepolymer surface was entirely sealed. The nutrient-deprived culture medium was directed out and the initial fed-batch synthesis is swapped to slowly progressing synthesis and constant harvesting between 7 and 10 days. A novel BNC was formed at the unoccupied surface of the synthesis medium. During its growth, the BNC fleece was continually transported through the bioreactor. The fleeces sizes can be adjusted by altering the speed of transport. The BNC was harvested through numerous belt conveyors. Once the BNC production has been started, the bioprocess could be kept continuous over some months, uninterrupted, and with no manual interference. Here, the steady-state condition was attained by continuous feeding of culture medium and air into the system. JeNaCell[®] is the BNC produced under HoLiR as a homogeneous, uninterrupted network assembly. It was reported to have 82–86% crystallinity when *G. xylinus* ATCC 11142 and *G. xylinus* DSM 14666 strains were used, respectively. This novel approach has significantly reduced not only the price of BNC synthesis, but also the time for starting, stoppage, and cleansing of the reactor in comparison to conventional static cultivation.

Static Intermittent Fed-Batch Technology

The cessation of bacterial growth and BNC production occurs in static production when the pellicle achieves a certain thickness. This occurs due to a cut down in the oxygen supply to the underside portion of the pellicle (which is in contact with the media) as the pellicle grows. This hampers the nutrient supply to the top portion, i.e., in the pellicle zone exposed to air (Dubey et al. 2018). This problem could be surmounted by supplying the media each time directly over the newly synthesized pellicle intermittently as shown in Fig. 6.

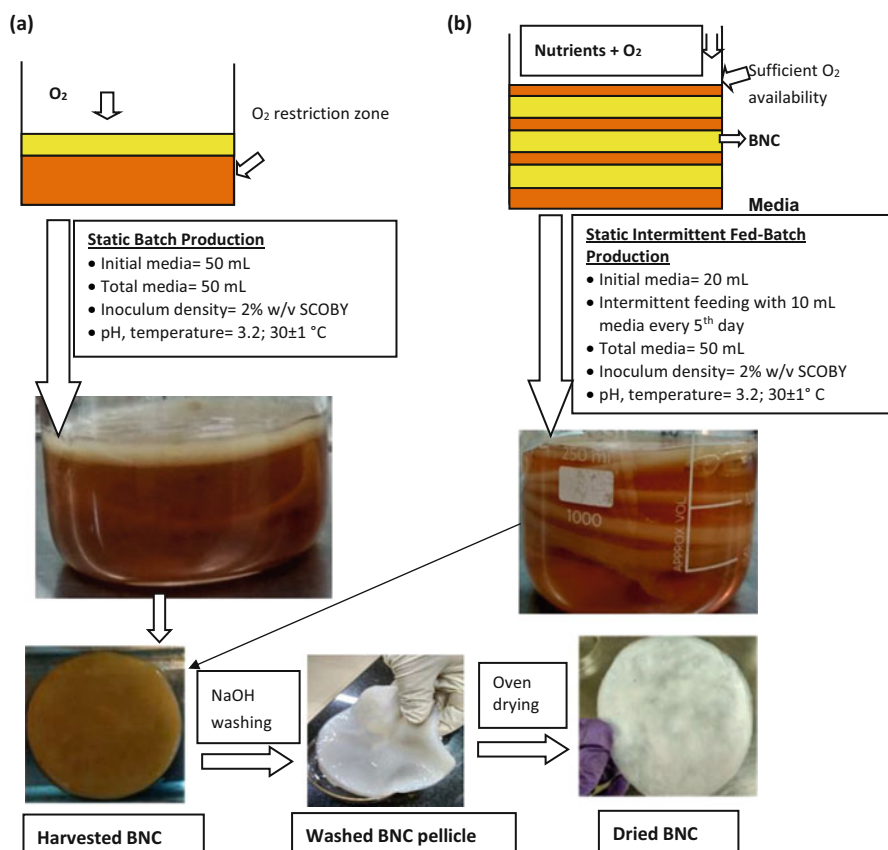


Fig. 6 Schematic showing BNC production using (a) conservative static method where media supplied only once resulted in oxygen reduction as BNC pellicle developed, thus blocking further BNC synthesis; (b) static intermittent fed-batch process with the addition of fresh media periodically, straightly over synthesized pellicle certified adequate oxygen and nutrients accessibility thus enhancing the BNC synthesis. (Modified and adapted from Sharma et al. 2021a)

Dubey et al. (2018) made use of static intermittent fed-batch technology for upgrading the BNC synthesis using a strain designated as *Komagataeibacter europaeus* SGP37. Here they made use of waste sweet lime pulp as a low-priced substrate. In conventional static synthesis, when the pellicle grows to a definite thickness, the nutrient and oxygen supply get reduced and slowly cut off leading to lesser BNC production. To defeat this constraint, fresh media was poured straightly over the formerly synthesized BNC pellicle periodically to uphold the high demand for oxygen and nutrient. Upon employing a static intermittent fed-batch process, the yield of BNC upsurged to 38 g/L from the initial yield of 26.2 g/L BNC as obtained through fed-batch cultivation (Dubey et al. 2018).

One of our recent studies (Sharma et al. 2021a) offers an industrially noteworthy strategy for the development of low-cost and eco-friendly BNC films using static intermittent fed-batch technology along with low-cost medium, i.e., fermented black tea broth. The outcome showed a creditable BNC formation of 29.2 g/L and 63.2% of substrate got converted to BNC which was nearly twofold higher than attained using the conventional static process (32%).

Cell-Free Extract Technology

Currently, a cell-free extract method (Fig. 7) has been discovered for the identification of the numerous intricate metabolic pathways leading to boosted production of BNC (Ullah et al. 2015). A cell-free system utilizes a series of enzyme-based biochemical processes to attain the transformation of the substrate to BNC. Here, the enzymatic, thermal, or mechanical treatments are performed to obtain a cell-free extract (Ullah et al. 2015). However, rupturing the *A. xylinum* culture mechanically by applying bead beating is one of the efficient, low-cost, and convenient methods for procurement of the cell-free extract.

Cell-free extract technology deviates from the conventional BNC synthesis techniques in the end phase (Islam et al. 2017). Here, the release of the chains of β -1,4 glucan to an exterior milieu skips the requirement of a distinct carrier scheme. The cell-free extract itself forms an exposed system where BNC can be collected directly onto the surface of media due to the disruption of the cell wall or membrane barrier. Thus, this process eradicates the requirement of enzyme-based bacterial transport machinery for the extraction of cellulose chain to the surrounding (unlike

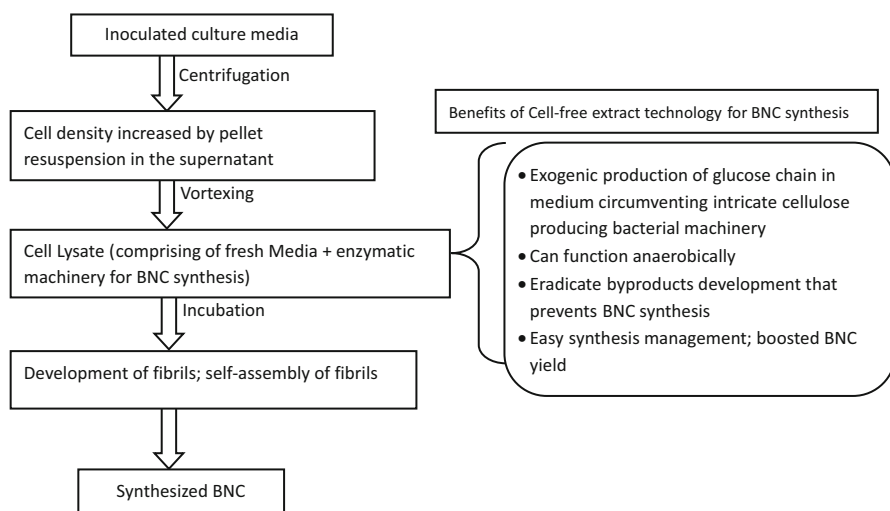


Fig. 7 Cell-free extract technique and its benefits over conventional BNC synthesis technique. (Adapted and modified from Sharma and Bhardwaj 2019a)

obligatory in conventional systems). In this way, this technique also aids in avoiding energy consumption in the form of adenosine triphosphate (ATP). Thus, large-scale BNC production can be achieved using this novel cost-effective metabolic engineering strategy. Therefore, the cell-free extract technique could emerge as an ideal impending method for BNC production. It was reported that the yield of BNC in the cell-free system was 57.68% in contrast to 39.62% in the traditional static system (Ullah et al. 2015).

Bioreactor-Assisted Approach

A bulk quantity of research on BNC synthesis was done using either the static or agitated synthesis approach. The BNC synthesis using alternate methods that might provide creditable BNC production has been investigated in a limited amount. There is scarce research on BNC production using alternative approaches (Sharma and Bhardwaj 2019a). Due to this research void, the effectual production of BNC has been hindered. The use of a bioreactor-based approach for BNC synthesis can offer a commendable BNC yield at an industrial scale; however, this necessitates additional study. The use of a bioreactor certifies rational regulation of nutritional and physiological parameters, thus assisting the appropriate growth of BNC producers. At present, scientists are focussing on increasing BNC productivity by exploring diverse categories of bioreactors (Chao et al. 2000; Krystynowicz et al. 2002; Campano et al. 2016). A prerequisite is to accomplish appropriate control of parameters during BNC synthesis. Consequently, a bioreactor that lowers the culture period and conserves the cultural authenticity is extremely desired for effective BNC synthesis. The bioreactors employed earlier for BNC synthesis have a simple design. Therefore, additionally complicated and unusual bioreactors are essential requirements for BNC synthesis. The augmentation in the BNC synthesis can be accomplished by fixing the production parameters like temperature, pH, and oxygen pressure to an optimum level throughout the fermentation process. Thus, *probing into customized bioreactors for BNC synthesis is still an open matter to upsurge BNC productivity for the envisioned practice.*

The current research emphasis is to enhance BNC throughput by utilizing diverse forms of bioreactors (Krystynowicz et al. 2002; Campano et al. 2016). Weyerhaeuser was the first company to investigate the production of bacterial nanocellulose called “cellulon” produced by stirred fermenters (Gama et al. 2016). Subsequently, numerous types of culture devices have been envisaged and investigated for BNC synthesis using agitated mode. These include stirred tanks, shake flasks, and modified airlift or gas-lift bioreactors. Sphere-shaped particles or fiber-like structures are usually formed within such devices. Figure 8 reveals few reactors employed for BNC synthesis so far.

Airlift or stirred tank reactors when used for BNC synthesis resulted in decreased yield due to the attachment of culture broth to the interior and upper side of the reactor (Krystynowicz et al. 2002).

Fig. 8 BNC produced in advanced rotary disc bioreactor: BNC deposited on both sides of discs



A reactor designed in the form of a cylindrical vessel escorted with a silicone membrane of distinct thickness was anticipated for BNC synthesis. Here the BNC pellicles were synthesized on the oxygen permeable sheath at the surface of the liquid. Its design resembles a cylindrical vessel and contains a silicone membrane of definite thickness at the bottom. The rate of nanocellulose production on silicone membrane firmly depends on the degree of roughness. A fivefold increase in synthesis rate was observed with the glossy surface as compared to the embossed surface (Yoshino et al. 1996). A twofold surge in BNC productivity was observed in a silicone membrane-based reactor by *A. pasteurianus* AP-1SK strain.

Other recognized examples are the linear conveyor film bioreactors and nonstatic rotating discs used by Bungay and Serafica (2000) for BNC synthesis. The BNC was synthesized in a tubular shape by Klemm et al. (2001).

The next reactor type was the aerosol bioreactor presented by Hornung et al. (2007). The aerosol generator used two removable and sterilizable chambers and operates by the production of a spray of the nutrient broth by ultrasound.

Another one is the rotating disk bioreactor (RDBR) which was designed so that the surface of the discs remained partially exposed to the nutrient broth and partly to the air. Consequently, during BNC synthesis, bacterial cells wedged to the disc's surface. They obtained nutrients from the medium and were subsequently remained in contact with the air. It was documented that the previous investigation using a self-constructed rotating disk system provided a nonhomogeneous mass of low-quality and mechanically weak BNC segments. Besides, the growth of cellulose in the substrate vessel has caused interference in the rotation of the discs. Here, the BNC was attached consistently to the mesh present on the discs. The tremendously promising environment for the BNC synthesis under a rotating disk bioreactor was studied by Krystynowicz et al. (2002), by shifting parameters like the number of discs, medium volume, and rotational speed. It was reported that the maximum BNC synthesis has occurred with a surface area to culture volume (S/V) ratio of 0.71 cm^{-1} at 4 rpm (Krystynowicz et al. 2002). BNC-synthesizing bacteria obtained an appropriate oxygen distribution and do not experience severe shear stress under rotary disc bioreactor-assisted synthesis. Kim et al. used a rotary disc bioreactor equipped with

eight discs for BNC synthesis using *Gluconacetobacter sp.* to produce 5.52 g/L BNC (Kim et al. 2007). However, it has a very primitive design and cultural parameters could not be controlled throughout the synthesis process.

BNC synthesis can be augmented by the addition of plastic composite supports (PCS) to the bioreactor (Cheng et al. 2010). The PCS-assisted bioreactor (10 L) produced 28.3 g BNC dry weight in 4 days of fermentation (Zahan et al. 2016).

Another type of reactor for BNC synthesis was designed with a spin filter. It has the following parts: a fermenter vessel, a six-bladed turbine impeller, and a spin filter containing a cylinder enclosed with a mesh of stainless steel and an agitator (Jung et al. 2007). It was reported to produce 2.9 times higher BNC yield than attained in a static fermenter in 140 h using *G. hansenii* PJK strain. Another reactor design includes a spherical-type bubble column bioreactor that abetted for obtaining huge BNC productivity, i.e., 1.9–2.27 g/L/day (Campano et al. 2016). It offered appropriate aeration and oxygen level and curtailed the shearing stress caused by agitation.

Our recent study investigated the advanced rotary disc bioreactor (RDBR) for BNC synthesis employing the strain *Gluconacetobacter xylinus* NCIM 2526 (Fig. 8) (Sharma et al. 2022). RDBR-assisted synthesis of BNC provided the wet weight of BNC equivalent to 189 ± 14 g/L, which corresponded to 6.6 ± 0.3 g/L dry BNC yield in 10 days. It was also investigated that under static mode only 56 ± 12 g/L wet weight of BNC, corresponding to 2.4 ± 0.4 g/L dry weight, was produced. RDBR has produced approximately 2.75-fold greater BNC as compared to static synthesis utilizing the equal volume of the media. The BNC got deposited on both sides of the discs during fermentation due to increased surface area provided by mesh present on the discs. It was found that the conversion yield of sugar to BNC was $12.2 \pm 0.8\%$, i.e., double the value obtained using static BNC synthesis ($6.2 \pm 1.4\%$) with effectual sugar consumption of $90.0 \pm 3.3\%$ (Sharma et al. 2022). It was the earliest description of the BNC synthesis in the advanced RDBR, using *Gluconacetobacter xylinus* NCIM 2526 strain. The pH and temperature were regulated throughout the synthesis process. This study might offer a productive technical approach for the resourceful synthesis of BNC.

Whereas improved BNC yield was obtained employing these reactor designs, there remains a dearth of planned experiments using different bioreactors. Bioreactor-based studies accompanied with appropriate conditions and economical substrates must be further researched to attain an exciting yield of BNC following the escalating industrial interest. Forthcoming research must emphasize BNC synthesis using bioreactor-assisted strategies in combination with different low-cost media and other bacterial strains and mixed cultures. Figure 9 presents some of the reactor designs used to date for BNC production.

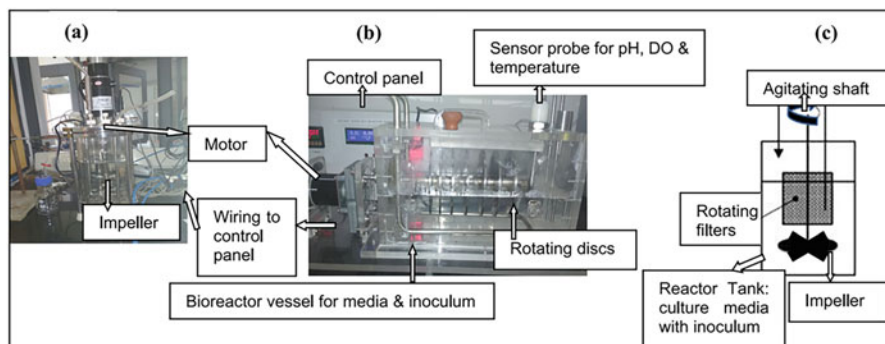


Fig. 9 Elementary plan of few bioreactors utilized for BNC synthesis. (a) Stirred tank reactor; (b) rotary disc bioreactor; and (c) bioreactor equipped with a spin filter. (Adapted from Sharma and Bhardwaj 2019a)

Scale-Up Strategies for BNC Productivity Enhancement by Surmounting Snags

Augmenting BNC Synthesis by Substrate-Based Strategies

The increasing industrial appliance for BNC and its derivatives in varied technological fields present a driving force for carrying out exhaustive studies for enhancing the synthesis strategies and reducing the synthesis cost. The right media for the synthesis of BNC is vital for economical synthesis and yield enhancement. The frequently used carbon sources include fructose, glucose, sucrose, mannitol, glycerol, etc. (Mohammadkazemi et al. 2015; Bungay and Serafica 2000; Hornung et al. 2007; Zahan et al. 2014). A typical Hestrin-Schramm media (HS media) used for the synthesis of BNC is overpriced (Lin et al. 2013) and entails supplementary kinds of stuff like yeast extract, glucose, peptone, and ethanol. So, one of the key hurdles clutching attention in BNC synthesis technology is to predict an appropriate cheap carbon source. Attempts have been made to procure cheap BNC by using an array of carbon sources, and additional kinds of stuff (Cheng et al. 2017; Dourado et al. 2016). An extensive range of substrates, supplements, and methods of BNC synthesis used till now, aiming to expand BNC synthesis, are stated in Table 3.

The BNC synthesis using alternate substrates like sweet lime pulp (Dubey et al. 2018), sugarcane molasses (Tyagi and Suresh 2016), fruit juices and rotten vegetables, sugarcane straw hydrolysates, paper mill sludge, etc. have been achieved so far (Jozala et al. 2016).

Molina-Ramírez and his team made use of a new bacterial strain, i.e., *Komagataeibacter medellinensis*, which was resistant to acid, for BNC synthesis utilizing low-cost substrates like food supply chain wastes and agricultural by-products unsuitable for human eating (rotten bananas, rotten mangoes, and cheese whey (Molina-Ramírez et al. 2018)). Various additives were included in the

Table 3 Studies representing BNC synthesis using varied substrates and techniques. (Modified and adapted from Sharma and Bhardwaj 2019a)

Production strategy	Microorganism	Medium for C source	Supplements used	Time	Yield (g/L)	References
Static	<i>A. xylinum</i> BRC 5	G	C ₂ H ₅ OH, O ₂	50 h	15.30	Hwang et al. (1999)
	<i>Acetobacter</i> sp. A9		C ₂ H ₅ OH	8 days	15.20	Son et al. (2001)
	<i>G. hansenii</i> PJK (KCTC 10505 BP)			72 h	2.50	Park et al. (2003)
	<i>A. xylinum</i> BPR 2001	F	Agar, O ₂	72 h	14.1	Bae et al. (2004)
	<i>A. xylinum</i> BPR2001	Mo	Different vitamins, inositol, riboflavin	3 days	0.14	Bae and Shoda (2004)
	<i>A. xylinum</i> NBRC 13693	Pineapple media	No	14 days	4.1	Kurosumi et al. (2009)
		Orange juice media			5.9	
	<i>G. xylinus</i> ATCC 53524	S	No	96 h	3.83	Mikkelsen et al. (2009)
	<i>G. sacchari</i>	G	No	96 h	2.70	Trovatti et al. (2011)
	<i>A. xylinum</i> 186		n-butanol	133 h	1.33	Lu et al. (2011)
	<i>G. xylinus</i> (BCRC 12334)		Thin stillage	168 h	10.38	Wu and Liu (2012)
	<i>G. persimmonis</i> GH-2	Ga + S	No	14 days	7.67	Hungund et al. (2013)
		Ga + L			6.89	
		Ga + M			6.28	
		Ga + F			5.82	
		HSM +			5.75	
		M			5.98	
		Watermelon			6.18	
		Orange juice				
		Muskmelon			8.08	
		Coconut water			5.75	

(continued)

Table 3 (continued)

Production strategy	Microorganism	Medium for C source	Supplements used	Time	Yield (g/L)	References
	<i>A. xylinum</i>	Banana peel		15 days	19.46	Khami et al. (2014)
	<i>G. intermedium</i> SNT-1	Heat pretreated molasses		10 days	12.6	Tyagi and Suresh (2016)
	<i>G. xylinus</i> ATCC-53582	Rotten fruit culture		96 h	60	Jozala et al. (2016)
	<i>A. xylinum</i> 23769	Hot water extract of wood		672 h	0.15	Kiziltas et al. (2015)
	<i>G. xylinus</i> CGMCC 2955	Candied jujube hydrolysate wastewater		144 h	2.25	Li et al. (2015)
	<i>G. xylinus</i> CH001	Wastewater after lipid fermentation	Citric acid	5 days	0.66	Huang et al. (2016)
	<i>G. xylinus</i>	CHbM		9 days	1.8	Bilgi et al. (2016)
	<i>Acinetobacter</i> sp. BAN1	PIWAM and PAWAM		15 days	0.4–0.6 (PIWAM) 0.2–1.1 (PAWAM)	Adebayo-Tayo et al. (2014)
	<i>A. pasteurianus</i> PW1				0.1–3.9 (PIWAM) 0.2–1.0 (PAWAM)	
	<i>G. xylinus</i>	Hydrolysate of durian shell		8 days	2.67	Luo et al. (2017a)
		Acid hydrolysate of waste oleaginous yeast biomass		4 days	2.9	Luo et al. (2017b)
		ABE fermentation wastewater		7 days	1.34	Huang et al. (2015)

	<i>G. xylinus</i> BC-11	Pullulan polysaccharide fermentation wastewater		10 days	1.177	Zhao et al. (2018)
	<i>K. rhaiticus</i> iGEM	Fermented tea		10 days	–	Florea et al. (2016)
	<i>Beijerinikia fluminensis</i> WAUPM53 and <i>Gluconacetobacter xylinus</i> 0416	Hydrolysate of sago by-product		14 days	0.4	Voon et al. (2019)
	<i>K. xylinus</i> strain K2G30 isolated from fermented tea	G	C ₂ H ₅ OH	10 days	23	Gullo et al. (2017)
Static and static intermittent fed-batch	Fermented black tea broth: symbiotic culture of bacteria and yeast	G and other sugars present in black tea	No	20 days	13.3 and 29.2	Sharma and Bhardwaj (2019b); Sharma et al. (2021a)
	<i>A. xylinum</i> BPR 2001	Mo		72 h	7.82	Bae and Shoda (2004)
Static/agitated	<i>G. xylinus</i> IFO 13773	G	Lignosulfonate	7 days	10.1	Keshk and Sameshima (2006)
	<i>A. xylinum</i> E25	G	C ₂ H ₅ OH (1%)	64 h	3.10	Krystynowicz et al. (2002)
Agitated	<i>A. xylinum</i> JCM 9730	G	Methylcyclopropane-I	288 h	1.20	Hu et al. (2013)
	<i>Acetobacter</i> sp. V6	Mo and CSL	CH ₃ COOH	168 h	3.12	Jung et al. (2007)
	<i>A. subsp. Suicrofermentans</i> BPR2001	CSL-fnu medium	No	40 h	8.0	Watanabe et al. (1998)
	<i>G. hansenii</i> CGMCC 3917	Waste beer yeast treated with ultrasonication		–	7.02	Lin et al. (2014)
Airlift reactor	<i>A. xylinum</i> subsp. <i>suicrofermentans</i> BPR2001	CSL-fnu medium		67 h	3.8	Chao et al. (2000)
	(continued)					

Table 3 (continued)

Production strategy	Microorganism	Medium for C source	Supplements used	Time	Yield (g/L)	References
Five-stage horizontal flow reactor	<i>A. xylinum</i> strain DA	G	CH ₃ COOH	68 h	0.15	Toda et al. (1994)
	<i>A. xylinum</i> 0416	Pineapple waste medium	No	4 days	28.30	Zahan et al. (2016)
Rotary disc reactor	<i>G. xylinus</i> NCIM 2526	HSM		10 days	6.6	Sharma et al. (2022)
	<i>G. sacchari</i>	Industrial residues from olive oil production		–	1.28	Gomes et al. (2013)

G glucose, *F* fructose, *Mo* molasses, *Ga* galactose, *L* lactose, *M* maltose, *HSM* HS medium, *CHbM* carob and haricot bean medium, *PIW4* pineapple waste medium, *PAW4M* pawpaw waste medium, *ABE* acetone-butanol-ethanol, *CSL*: corn steep liquor, *CSL-fru* corn steep liquor-fructose

media for upsurging BNC production such as alcohols (Mikkelsen et al. 2009), organic acids and vitamins, agar (Reiniati et al. 2017), carboxymethyl cellulose corn steep liquor (Reiniati et al. 2017), etc.

BNC synthesis was reported by other substrates like residues from the wine industry (Cerrutti et al. 2016). Ethanol is reported to be a very efficient additional carbon source. It was observed that there is an increment of 2.31 g/L from 1.30 g/L in BNC yield by *G. hansenii* when the culture medium was supplemented with ethanol. In another study, a fourfold increase in BNC productivity was observed by *Acetobacter* sp. A9 strain when ethanol was added to the medium (Son et al. 2001). Another supplement is reported to augment the BNC yield by 0.4% in *K. xylinus* BPR2001 (Bae et al. 2004). It was observed that agar addition offers increased viscosity to the medium which has influenced BNC yield (Kim et al. 2012).

Replacement of water with thin stillage (a rice wine distillery wastewater) boosted the BNC yield by 2.5 times (Wu and Liu 2012). When the constituents of sugarcane molasses such as sucrose, glucose, fructose, nitrogenous compounds, agar, nonnitrogenous acids, and vitamins were added to the HS medium, the BNC yield got upsurged with *Acetobacter xylinum* ATCC 10245 strain (Premjet et al. 2007).

A study showed that a healthy drink of Chinese origin, i.e., kombucha (fermented black tea) sweetened with glucose under static conditions, resulted in the synthesis of BNC film adhering to the air-liquid interface of the culture medium. A symbiotic culture of bacteria and yeasts (SCOBY) is known to start the fermentation of tea (Fig. 10) (Sharma and Bhardwaj 2019b). Owing to the typical occurrence of vital nutrients and growth supplements like polyphenols, ethanol, vitamins, etc., fermented black tea can serve as a low-priced medium in comparison to conventionally available media (Jayabalan et al. 2014). *Gluconacetobacter xylinus* ZHCJ618 was known to be isolated from kombucha and indorsed for marketable usage owing to its virtuous stability in culture media and reasonable synthesis of BNC, i.e., 7.56 ± 0.57 g/L and 8.31 ± 0.79 g/L under static and shaking conditions, individually (Zhang et al. 2018). Different parameters of BNC synthesis using fermented tea have been evaluated and it was observed that 90 g/L sugar concentration added to the black tea provided the maximum productivity of 66.99% in 8 days (Goh et al. 2012). In a study, the glucose-supplemented fermented black tea broth was employed as a cost-effective medium for BNC synthesis, providing 13.3 g/L BNC yield (Sharma and Bhardwaj 2019b). Fermented black tea along with glucose serves as a complete media without the necessity of supplements which are normally added to the conventional media. Furthermore, the study revealed that brewed tea (waste remained after tea making) can also offer a virtuous yield of 12.8 g/L BNC. Such a low-cost medium, if used sideways with altered strategies, can bring innovations in the area of acquiring low-priced BNC. Additionally, this research work showed that the assorted culture of microorganisms might be valuable in enhancing BNC production in comparison to an individual conventional strain (Sharma and Bhardwaj 2019b). Thus, one of the cheapest media for the synthesis of BNC might be the fermented tea broth (Kombucha).

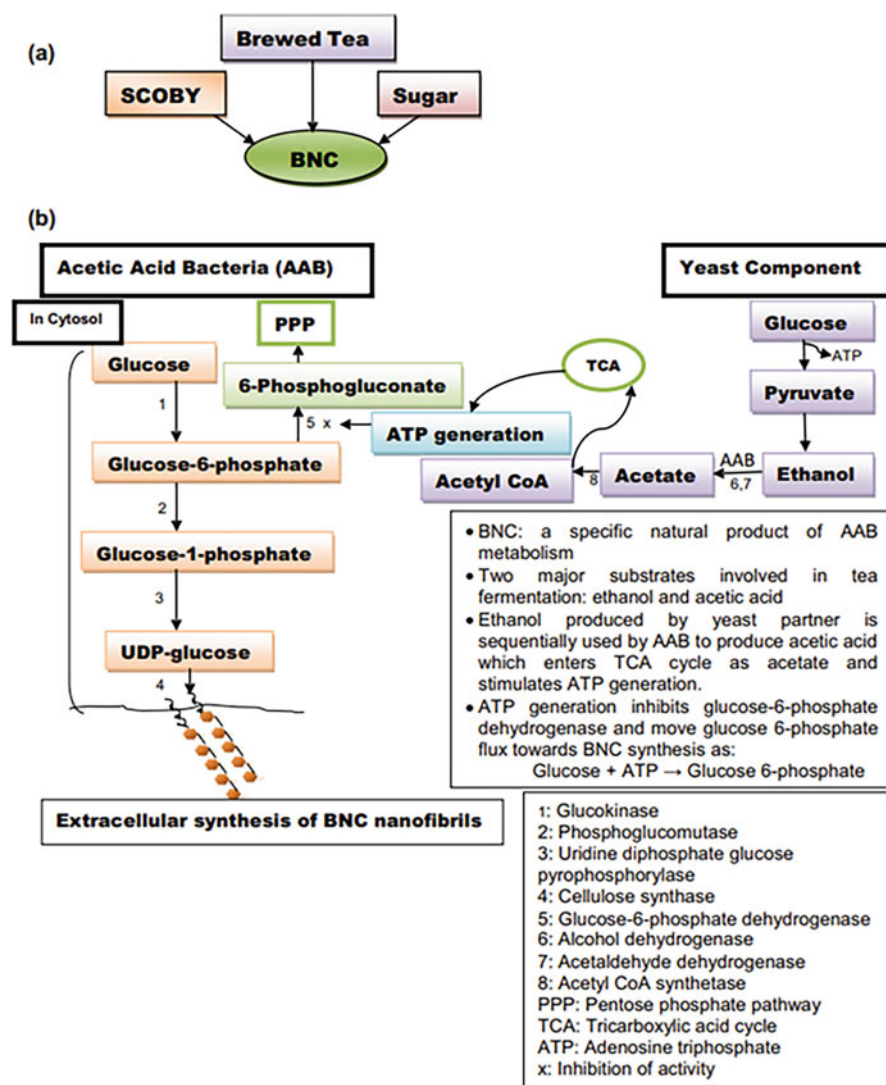


Fig. 10 (a) Three essential components for BNC production in FBTB; (b) understanding dynamic interplay of SCOBY in FBTB directing BNC production. (Adapted from Sharma and Bhardwaj 2019b)

Coconut water is the next vital substrate for BNC synthesis. Nata de coco, the first accessible edible BNC product, resulted from the fermentation of coconut water via acetic acid bacteria (Jagannath et al. 2008). This work permitted the resourceful consumption of coconut water, a hitherto wasted by-product of the coconut industry as a means of procurement of value-added products for other industries. Onggo et al. 2015 used coconut water to form BNC (Nata de coco). Additionally, they were

reported to impart magnetic properties to the BNC for various use (Onggo et al. 2015). Coconut water comprises worthy nutrients for *Acetobacter xylinum* such as proteins, soluble sugars, few oils, salts, and vitamins and it mainly comprises sugar as a topmost portion of soluble solids. Coconut skim is the remnants of the virgin coconut oil synthesis process and is customarily discarded of causing environmental pollution. Coconut skims having countless dietary values in form of protein, vitamins, and minerals could be employed as a medium for BNC synthesis. It was the first report on the fruitful consumption of coconut skim as a constituent of media for BNC synthesis (Andasuryani et al. 2017).

In-depth research with substituted economical carbon sources is obligatory in the pursuit of searching for appropriate substrates for superior BNC synthesis. Accordingly, the usage of substituted affordable media accompanied by altered synthesis processes as well as innovative BNC synthesizing strains (other than the typically used strains) must be the emphasis of imminent research to acquire worthy BNC synthesis for its widespread usage.

Strain Improvement for Large-Scale BNC Production

Extensive research has been done to augment BNC production at an industrial scale as well as to curtail the BNC synthesis charges, such as by employing low-cost media and finding substitute carbon sources and supplements. However, one of the aspects which remained much untapped is the BNC biosynthesis mechanism itself to enhance productivity and yield and to tailor the basic structure of BNC to meet the necessities of definite applications (Manan et al. 2022). The molecular studies showed the contribution of specific operons (bcsABCD) in the BNC biosynthesis, extracellular transport, and in vitro supramolecular assembly of cellulose fibrils. It has been revealed that BNC is synthesized by definite operons leading to cellulose synthesis. This involves two homologous functional elements, the BNC synthesis operon (bcsABCD, type I) and acetobacter cellulose synthesis (acsABCD) operon, which translate crucial proteins for BNC production by *A. xylinum* ATCC 1306-3 and *A. xylinum* ATCC 53582 (*K. xylinum*), individually. The bacterial genome alteration can be accomplished by genetic recombination or gene mutation through an outside chemical or physical stimulus called mutagen. The effective reports on genetic alteration of some bacterial strains are intended for increased yield and productivity, as well as altering the BNC structure. Numerous genes also augment the biosynthesis of BNC producers. For example, sucrose synthase, which is encoded by the sucrose synthase (SS) gene that catalyzes the reversible translation of sucrose, fructose, and UDP-Glc in plants, is also known to boost BNC production. In research, genetic alteration of the *K. xylinus* BPR2001 strain with a disrupted SS gene from the Mung bean led to BNC biosynthesis augmentation up to threefold as compared with its wild-type counterpart. Battad-Bernardo and coworkers developed a recombinant *K. xylinus* ITz3 strain by incorporating the transposons into the chromosomal DNA, which formed a high concentration of β -glucosidase. The resultant mutant strain utilized lactose from whey as a carbon source and produced

28-fold more BNC than the wild strain (Battad-Bernardo et al. 2004). In a recent study, a genome-scale metabolic model (GSMM) of *K. xylinus* DSM2355 was formed using genome annotation data and metabolome study of the strain. This involved the overexpression of two genes, namely *pgi* (glucose6-phosphate isomerase) and *gnd* (phosphogluconate dehydrogenase) from *E. coli* or *Corynebacterium glutamicum*. The improved strain resulted in the synthesis of 3.15 g/L of BNC, which was about two times higher than the unmodified strain (1.46 g/L) (Jang et al. 2019).

BNC production was enhanced by around 40 and 230% in static and agitated cultures of the *Gluconacetobacter xylinus* strain, individually, by homologous replacement of the membrane-bound glucose dehydrogenase (GDH) gene with a deficient one (Kuo et al. 2016). The GDH system was known to be accountable for the wasteful conversion of medium glucose units to gluconic acid at the beginning of the incubation period. Jang and his coworkers reported that they attained the finest enhancement in the BNC yield by introducing the *pgi* overexpression gene heterologously to the strain *Komagataeibacter xylinus* 2325, which recorded about 3.15 g/L of BNC production and a 115.8% higher yield than the wild-type strain (1.46 g/L) (Jang et al. 2019).

Gamma ray has occupied the widespread attention of researchers due to its implications in genetic alteration for either augmented product yield or enhanced properties. Though it was selected as a pervasive disinfecting agent, the treatment of live cells by low dosages of gamma irradiation was described to attain exciting mutations. Besides, using gamma ray irradiation is economical and plausible in most research areas (Al-Hagar and Abol-Fotouh 2022).

BNC-Allied Challenges and Future Perspectives

Can bacterial nanocellulose substitute the traditional cellulose sources? It is enormously confrontational to discourse this query up to now. This question will remain unrequited till the scale-up strategies of BNC for marketable practice and fermentation bioprocesses to obtain reasonable BNC become furthermore innovative. The goal of prudently viable BNC synthesis has not been yet attained efficaciously despite plentiful studies executed on BNC synthesis. Figure 11 reflects the snags allied to efficacious BNC synthesis and its utility. The BNC-associated obstacles can be categorized into four key headings, i.e., synthesis-based, substrate-based, strain-based, and clinical advancement and marketing of BNC-based therapeutics.

Synthesis-Based Challenges

Most of the studies for BNC synthesis had been executed using either the agitated or static method. To date, a restricted quantity of bioreactor-assisted research has been performed. Thus, bioreactor-assisted BNC synthesis is required to be executed to certify appropriate regulation of culture conditions for the cutting-edge BNC

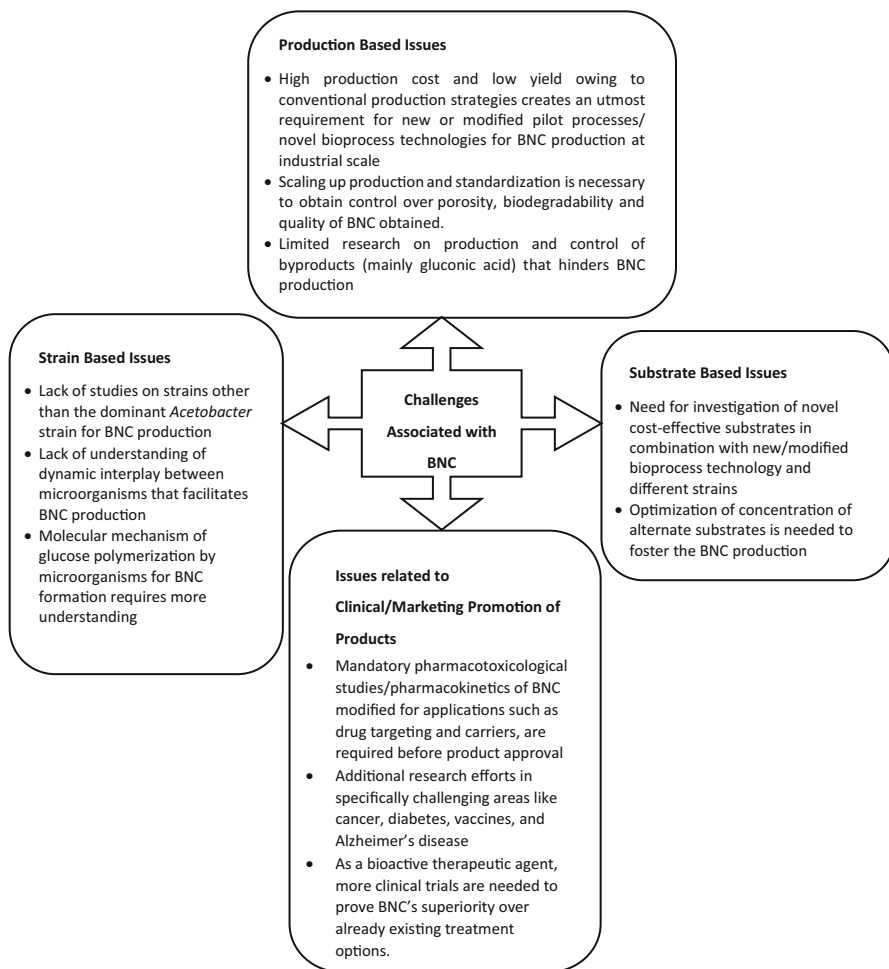


Fig. 11 BNC: challenges to be surmounted. (Modified and adapted from Sharma and Bhardwaj 2019a)

synthesis. At present, a very basic type of bioreactors has been employed for BNC synthesis. A potential BNC synthesis demands a more complex and advanced designed bioreactor. In an advanced bioreactor, the maintenance of pH and temperature of a bioreactor throughout the fermentation process could be attained using a pH probe and water jacket. This might bring an increment in BNC yield. The upgraded synthesis mode like cell-free extract system and static intermittent fed-batch strategy should be implicated with different agro/industrial wastes and strains to discover novel and germane union of technique, substrate, and strain for substantial BNC synthesis.

Substrate-Based Challenges

Approximately, one-fourth of the entire synthesis cost depends on the culture media, i.e., the substrate used for BNC synthesis. Thus, the pursuit of finding novel lucrative substrates which can contribute toward superior BNC yields in less duration is quite an arduous job for the reasonable BNC synthesis. Agro/industrial wastes have recently emerged as one of the profitable substrate groups being low cost. Studies utilizing these substrates must also be executed under precise conditions in advanced bioreactors to certify worthy BNC productivity. Furthermore, the application of agro/industrial wastes is likewise appreciable as it eradicates the wastes by transforming them into value-added products like BNC.

Strain-Based Challenges

To date, *Acetobacter* is the prevalent genus and a wide number of BNC synthesis research have been accomplished using *Acetobacter*. The application of mixed-culture strains of *Acetobacter* or other bacteria and yeasts along with low-cost agro/industrial-based media might enhance BNC synthesis. Attempts have been made in the last 20 years for isolating efficient BNC synthesizing strains. The pursuit of novel microbial strains either individually or in symbiotic associations that can synthesize BNC must be the emphasis of impending studies. So far, there are limited published data on the fruitful conversion and reprogramming of BNC-synthesizing bacterial strains. There is inadequate accessibility of plasmids (expression systems) of genes in biosynthetic pathways and regulatory networks for optimized BNC synthesis. Moreover, many available microorganisms such as *K. hansenii* ATCC 53582 are reported to have a low transformation rate. Therefore, this aspect must be comprehensively needed to be investigated for improving the BNC synthesis (Manan et al. 2022).

While there are prominent pathways for the synthesis of uridine diphosphoglucose (UDPGlc – a cellulose precursor), the molecular mechanism of polymerization of glucose into unbranched long chains necessitates further study. Therefore, studies to explore glucose polymerization at the genetic level might open a path to boost BNC synthesis in reduced time. Strain improvement and metabolic engineering might be the other approaches for enhancing the BNC synthesis. The assembly of toxic metabolic by-products often occurs during the industrial-scale BNC synthesis. The formation of gluconic acids as a by-product during the BNC fermentation had been experienced as one of the factors that negatively affect BNC synthesis (Pa'e 2009). Moreover, a noticeable trend is the conversion of cellulose-synthesizing strains to noncellulose synthesizers under oxygen-rich conditions inside the fermenters. The strains with decreased tendency to form gluconic acid during fermentation could be designed by strain improvement employing selection and mutagenesis. No definite data has been reported till date revealing the quantity of the gluconic acid formed during a fermentation process leading to BNC synthesis. Thus, studies to evaluate the amount of gluconic acid formed by different strains

during BNC synthesis might offer a more clear pathway for enhancing productivity. This could be attained by selecting the strains and carbon sources that produced a low amount of gluconic acid. The doubling time of *G. xylinus* is more as compared to *E. coli* and *B. subtilis*. Bacterial strains can be genetically modified to lessen the doubling time. Identification and isolation of strains that can readily be modified by genetic engineering to produce high-quality BNC with low-cost substrates must be investigated (Gama et al. 2016). The low-nitrogen-demanding strain of *Komagataeibacter rhaeticus* (*K. rhaeticus* iGEM), which is highly resistant to toxic chemicals, has been recently identified (Florea et al. 2016). As this strain can grow well under nitrogen stress, this property allowed its cultivation under cheaper, low-nitrogen-containing media. This has resulted in diminishing the synthesis cost. The team also succeeded in maintaining an external control over BNC production by *K. rhaeticus* iGEM, through the development of a modular genetic response that enables balanced reprogramming of the bacterial cell (Florea et al. 2016). Genomic sequencing of this strain was performed to reveal genetic circuits accountable for the functionalization and determining heterologous genetic expression within the BNC matrix (Florea et al. 2016). This cutting-edge discovery has set the basics for exploring genetic engineering for the synthesis of cellulose-based nanomaterials with versatile applications as biomaterials in basic sciences, nano-biocomposites designing, etc. The inclusive genetic information about diverse BNC-producing species followed by their metabolic modeling can provide the quality optimization of the BNC synthesis process. The scarcity of available genetic data creates an interruption of the utilization of this strategy for BNC synthesis. Thus, searching for the genomic sequence of the BNC synthesizers is of utmost necessity to evaluate the resemblances and variances at the gene level (Gama et al. 2016). Genome sequencing is beneficial in producing recombinant strains that might have the potential to produce a worthy amount of BNC with tailorable properties for diversified usage.

If the direct management of gene coding for the catalysts aiding BNC synthesis, regulatory protein machinery, and other genes directly or indirectly involved in the BNC synthesis could be attained, then this could create a remarkably promising step toward obtaining an industrially valuable bacterial strain. Nevertheless, BNC synthesized using genetically modified bacterial strains might have regulatory issues, mainly in the food and medical industries. Moreover, owing to the complicated regulatory mechanism involving each gene expressing its proteins with definite functions, changes at the molecular level may not certainly lead to a positive effect on the productivity of the synthesized BNC.

Besides this, more research must be executed by using microorganisms other than the dominant ones as these despite being present in a subsidiary amount might offer a required boost-up strength for starting the fermentation processes leading to BNC formation (Gama et al. 2016; Keshk and El-Kott 2017).

Clinical Advancement and Marketing-Related Challenges

BNC-based products and their marketing face a variety of obstacles at the clinical study level. Perhaps the greatest complex matter is to interpret the outcomes of in vitro analysis and animal-based studies into clinical applications on humans and make them marketable. Many BNC-based therapeutics are available; however, only a few have been approved for patient usage. The chief explanation for only a handful of the approved product lies in the snags at the manufacturing level and synthesis price. Moreover, the sterility problems and patent apprehension also restrict the products from being used for patient well-being.

Conclusions

BNC is an industrially significant biomaterial having wide usage in diverse high-tech applications. Thus, persistent efforts have been made for the viable acquisition of BNC by cutting down the synthesis price, yield enhancement, and comprehensively enlightening its performance by modifying the biological, physical, and mechanical properties. Currently, the three crucial strategies commonly applied for enhancing the BNC synthesis are: (i) reconnoitering the pioneering or altered synthesis tactics with both static and agitated modes like static intermittent fed-batch strategy, manipulative cell-free extract system, and employment of innovative bioreactors like advanced rotary disc bioreactor; (ii) substitution of conventional culture media with a low-cost agricultural/industrial wastes to bring down the synthesis cost; and (iii) genetic alteration of microorganisms used for BNC synthesis. Besides this, studies must be conducted using other possible strains in addition to the dominant BNC synthesizers.

There is no efficient and automated mass production process for industrial-scale BNC synthesis, whereas numerous BNC-based products are commercially available that predict the status of BNC in the development of bio-based products. Thus, BNC is presently not economically competitive in comparison to other more recognized polymers in most applications. Nevertheless, the designing of new technical processes and their optimization are ongoing to obtain diverse achievable forms of BNC. For the synthesis of flat BNC in the fleeces and foils forms, novel reactor types have been developed to overcome the most important drawbacks of the static method. The (semi) continuous HoLiR method has been recently shifted to synthesis at a pilot scale. The rotary disc bioreactor-assisted synthesis has been successfully carried out to enhance the BNC production. All these signs of progress have reinforced the anticipation for future large-scale synthesis processes, which is instantly desirable to bring the auspicious BNC-based products from the laboratory to the market. Moreover, the advanced bioreactor designs and techniques like static intermittent fed-batch and cell-free extract, if investigated with different culture media, might build a road to substantial and economical BNC production. To summarize, it can be concluded that the reasonable utilization of BNC depends on its yield. Thus, if BNC can be effectually manufactured in a plentiful amount at a low

cost, it can be used in the production of various biomedical devices and other consumer products. If a further alteration in bioprocess technology such as bioreactor design, process parameters, and employment of cheap or wastes substrates as media components could be attained, it would undoubtedly assist in acquiring a high-quality nanocellulose. *Thus, the factors, that need further investigation for commendable BNC synthesis, are the appropriate combination of the production strategies, substrates, and strains.*

Cross-References

- ▶ [Bacterial Nanocellulose \(BNCs\) Supported Inorganic Nanomaterials for Catalytic Applications](#)
- ▶ [Bacterial Nanocellulose from Agro-industrial Wastes](#)
- ▶ [Biocompatibility of Nanocellulose](#)
- ▶ [Biopolymers for Edible Films and Coatings in Food Applications](#)
- ▶ [Packaging Applications of Biodegradable Nanocellulose Composites](#)
- ▶ [Tunable Biopolymers](#)

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Bacterial Nanocellulose from Agro-industrial Wastes

4

Heru Suryanto, Uun Yanuhar, B. Brailson Mansingh, and J. S. Binoj

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H. Suryanto (✉)

Center of Excellent for Cellulose Composite (CECCom), Faculty of Engineering, Universitas Negeri Malang, Malang, Indonesia

e-mail: heru.suryanto.ft@um.ac.id

U. Yanuhar

Department of Aquatic Resources Management, Brawijaya University, Malang, Indonesia

e-mail: doktoruun@ub.ac.id

B. B. Mansingh

Sri Ramakrishna Engineering College, Coimbatore, India

e-mail: sweetbrailson@gmail.com

J. S. Binoj

Institute of Mechanical Engineering, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India

e-mail: binojlaxman@gmail.com

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Abstract

The transition from an oil-based economy to a nature-based economy necessitates the innovative exploitation and transformation of high-value natural resources. Cellulose is mostly found in higher plants, but it is also created by marine organisms, particularly Tunicates, types of algae, and bacteria, by enzymatic and chemical processes, with a nanocellulose or nanofiber or nanocrystalline cellulose structure. For environmental sustainability, it would be preferable to use natural fibers as a renewable source as the worldwide waste problem caused by biomass is becoming an increasing environmental concern. Numerous agro-industrial wastes have been investigated for the synthesis of Bacterial cellulose (BC). Bacterial cellulose fiber is a natural fiber that is easy to cultivate and offers nano-sized fiber. BC pellicle can be created using two types of fermentation: dynamic and static fermentation using Hestrin and Schramm medium with carbon sources derived from agro-industrial waste. Bacterial Nanocellulose (BNC) is extracted through the fibrillation of BC pellicle in a high-pressure homogenizer, which reduces and homogenises the particle size. It is applicable as composite membranes or hydrogels. The X-ray diffraction, fourier transform infrared, scanning electron microscope, and strength analysis can be used for characterization. Bacterial strain and fermentation procedures determine the BC's properties. Small-scale and contemporary enterprises have utilised BNC for biomedical, energy harvesting and storage, electrical, environmental, and food applications. This chapter presents a review of agro-industrial waste, cellulose feedstock, bacterially produced cellulose fiber, its morphological, chemical, physical, and mechanical characteristics, as well as its potential applications.

Keywords

Agro-waste · Bacterial Nanocellulose · Biosynthesis · Cellulose · Membrane · Homogenizer

Introduction

Cellulose is the earth's most abundant substance with low density and excellent mechanical characteristics. An estimated 10^{10} to 10^{11} tonnes are extracted annually from plants or garbage on a global scale (Figueiredo et al. 2014). Cellulose is mostly found in higher plants but is also created by marine organisms, such as Tunicates, algae, and bacteria, by enzymatic and chemical processes (Musa et al. 2016). Multiple industries are predicted to account for the majority of cellulose consumption in the current period. With a tensile strength of 16.9 MPa and a cellulose modulus of 2 GPa (Gallegos et al. 2016), cellulose is extensively utilized as a material for rayon fiberboard paper in the manufacturing business. Plant cellulose is a combination of carbohydrates, including hemicellulose and lignin, which weaken the cellulose fibers. As a bulking agent and fat substitute in low-calorie foods, many kinds of cellulose, such as texture modifiers, emulsifiers, and extenders, can be utilized. In food items, it is derived as a pulp from fibrous materials such as cotton or wood. It is a reinforcement for composite materials, biomedical implants, hydrophobic polymer matrices, and medicinal applications. Numerous researchers employ cellulose in the form of nanofibrils or nanocrystalline cellulose as a polymer composite filler. This nanocrystalline cellulose is recovered from plant fibers by crushing them and then subjecting them to an acid hydrolysis procedure to destroy the amorphous fraction and obtain crystalline cellulose. However, this procedure is costly due to its high energy requirements. Therefore, a method for manufacturing nanocellulose from bacteria was devised to facilitate the replacement of plant cellulose nanofibrils (Esa et al. 2014) and contribute to the environmental sustainability of plants.

Numerous research has discovered applications for lignocellulosic biomass that can be utilized as a starting point for the manufacture of other substances, such as biofuels and biopolymers. The primary sources of lignocellulosic waste are now classified as agricultural, forest, and industrial resources. As the main component of lignocellulosic waste, cellulose is a natural polymer consisting of glucose duplication units with unbranched chemical chains. Alternative therapies utilize the waste from argan-based cakes, olive tree pruning, cotton buds or apple pomace and grapefruit, as well as nanocrystals and cellulose nanofibers. Many sectors, including food packaging, biomedicine, mechanical reinforcement, and membrane filters, have used nanocellulose-based materials into their products. Nanocellulose has recently been the focus of a great deal of study about goods and applications, including nanocomposite, foams, fibers, barrier layers, films, viscosity fixes, gels and aerogels, energy consumption, and filters. Depending on the processing conditions, nanocellulose can be extracted from Lignocellulosic biomass by dissolving cellulose fibers into cellulose nanocrystals and cellulose nanofiber. The most common techniques for detecting cellulose nanocrystals and cellulose nanofibers, respectively, are acid hydrolysis and mechanical treatment; however, these processes have some shortfall related to environmental and economic factors, such as the high amount of water required for neutralization.

Enzymatic hydrolysis is a viable natural and ecological alternative to nanocellulose manufacturing due to the benefit of enzymatic treatment, while the enzyme cost can be low. Several authors have investigated the utilization of enzymes in conjunction with mechanical treatment to promote the formation of cellulose bonds and lower the energy demands of nanocellulose separation procedures. Nanocellulose is a general term that is often used for various nano-sized cellulose particles. Depending on the structure, nanocellulose is the result of distributing cellulosic fibers to a wide range and length. There are three forms of nanocellulose: cellulose nanofibers, which are typically obtained through enzymatic and/or mechanical dispersion processes; crystalline cellulose-shaped nanocrystals, which are typically obtained through hydrolysis containing concentrated mineral acids; and bacterial nanocellulose, which is almost exclusively found in the bacterial family known as *Gluconoacetobacter xylinus*. The fundamental disadvantage of producing cellulose nanostructures is the significant energy consumption at high repetition rates, notwithstanding the essential breakdown cycles of biomass structures. Efforts to lower this energy use and achieve a more effective dispersion have been bolstered.

Microorganisms, such as bacteria, are capable of generating bacterial cellulose (BC) pellicle in the form of nata by polymerizing glucose molecules into α -1,4-glucan chains within the BC core (Iguchi et al. 2000). *Acetobacter xylinum* is the prevalent BC that ferments coconut water to make nata de coco. Under static fermentation conditions, these bacteria produce pellicles with exceptional features, including high crystallinity, high purity, exceptional mechanical capabilities, and the ability to build uniform membranes. These nano-sized sheets are biocompatible and feature a delicate structure. In addition, BC has good mechanical qualities (high tensile strength) and biodegradability. It has several applications as a biomaterial in the cosmetics and pharmaceuticals industries, as well as the paper, textile, and food industries, and can be utilized in various polymer science research.

The transition from an oil-based economy to a nature-based economy necessitates the innovative exploitation and transformation of high-value natural resources. The potential for converting natural waste into valuable natural materials is substantial. Agricultural biomass wastes consist primarily of burned or discarded fruit peels, roots, leaves, crop stalks, and seed shells. In practice, biomass wastes are a viable source of important feedstock materials. Globally, agriculture/forest industries create around 4.6 gigatonnes of wood biomass and almost 923 gigatonnes of trash (Tripathi et al. 2019). These wastes have the potential to harm the ground and surface waters and cause eutrophication. When contaminants are introduced into the soil, the microflora in the biomass is stimulated to generate and emit greenhouse gases, resulting in a worsening of global warming. Due to the varying chemical compositions of plant cellulose fibers, nanocellulose extraction is a complex and time-consuming procedure that increases processing costs. Moreover, the extraction of plant cellulose might contribute to global warming. Therefore, alternate cellulose sources, such as bacterial fermentation, are necessary.

Agro-Industry Waste

In the past 50 years, agricultural production has more than quadrupled due to the development of area for agriculture, the contribution of technology that influences productivity, and the accelerated growth of the world's population. Globally, agriculture produces as much as 23,7 million tonnes of food every day. Increased agricultural production exerts increasing strain on the environment, hence posing a danger to the sustainability of the ecosystem, depleting land, water, and air resources, and negatively impacting population health (Duque-Acevedo et al. 2020). Waste is defined as all non-optimally utilized materials, including manufacturing and consumption byproducts. Biodegradable waste contains a variety of chemicals, but they can be categorized into three primary constituents: hemicellulose, cellulose, and lignin. In contrast, garbage is typically the product of human activity that cannot be avoided and is therefore inexpensive. This comprises agricultural, industrial, municipal, and crop residues. It consists of plant materials, industrial, agricultural, and municipal wastes as well as residues. These include industrial and urban wastes, agricultural and plant products, and residues (Esa et al. 2014). Inefficient food processing results in the loss of food through spillage, spoilage, the discard of substandard edible ingredients, and the disposal of edible food components. Food waste has a considerable economic, environmental, and public health impact (Sundarraj and Ranganathan 2018).

The three categories of agro-based industries are non-food agro-industrial, animal and plant-based wastes. Except for the textile and leather tanning sectors, non-food-based agro-industries produce primarily biodegradable, non-toxic waste. The majority of garbage is left unutilized or untreated, posing a threat to the environment and human and animal health. The components of plant-based waste are process residues and field residues. Processed residue is the waste formed by the processing and refining of raw materials. Common crop-based agricultural wastes include products from fermentation-based industries, the sugar sector, grain flour mills, food and fruit processing. A substantial amount of organic residue from the poultry, meat, and fish processing industry contains proteins that are susceptible to releasing offensive odors and disturbing the public. Field residues are the waste items (stems, pods, stems, and leaves) left in the field after harvesting the crops. This waste is commonly utilized as bedding and animal feed for cattle, as well as for horticulture purposes (Singh et al. 2021). Agriculture, as the largest biological sector that produces the most biomass, represents a significant opportunity as an important input for the bioeconomy, not only due to their utilization and exploitation can reduce the use of fossil fuels and greenhouse gas emissions, but also because they contribute to the development of new jobs and green markets, and promote the conversion of lignocellulosic waste into value-added products such as food, bioenergy, bioproducts, and feed. The majority of agro-industrial waste consists of hemicellulose, cellulose, and lignin and is in the form of wet biomass. They can be exploited as a permanent source for the manufacture of compounds with added value (Singh et al. 2021).

Industrial products or food scraps produced by food processing, furniture design, printing and paper production, printing and packaging, such as sweet lime waste,

bagasse, and recycling paper, are a significant form of recycled material biomass used for nanocellulose extraction. In reality, the food processing industry alone will generate more than 142 million tonnes of cane trash year in Brazil, leaving a vast quantity of cellulosic waste with potential application in high value-added production. Due to feedstock's chemical and structural heterogeneity, it varies and is highly reliant on the type of fossil. With its high cellulose content and loose structure, sugarcane bagasse is amenable to acid hydrolysis for nanocellulose extraction. Prior to chemical extraction and acid hydrolysis methods, pulping and cleaning will be required in the case of nanocellulose extraction in the recycled paper or solid waste and waste pack industries.

The procedure of smoke explosion and acid hydrolysis is proposed to remove nanocellulose from the furniture industry for residual processing. Nanocellulose with a width between 18 and 40 nm was treated to surface rotation and ice suspension to produce the hydrophobic aerogel for oil absorption. Using the NaOH / Thiourea system, raw paper is purified and turned into circular nanocellulose with a crystalline index of 48.85% and a standard size of 50 nm for a specific type of industrial residue. The use of ammonium persulfate under oxidizing circumstances to convert fibers in paper mills and pulp into crystalline cellulose nanoparticles 10 to 20 nm in width and 150 to 500 nm long is described in another study. Using a catalyst, inorganic nanoparticles were produced. For antibacterial and photocatalytic operations in paper mills or as an additive for water treatment and textile finishing, sol-gel-prepared cellulose nanocrystals. Enzymatic hydrolysis is a novel technique that can facilitate the extraction of nanocellulose from industrial fossil fuels that produce a soft and loose cellular structure, such as industrial fossil-producing leftovers like citrus pulp.

In addition to industrial nanocellulose emissions and biomass residues, the use of bacterial nanocellulose implants in water processing businesses is a viable alternative for producing nanocellulose from inexpensive biomass. Researchers have transformed lime pulp waste into high-quality bacteria nanocellulose using *Komagataeibacter europaeus* SGP37 with fermentation process in a static intermittent batch, establishing a novel method for transforming trash into high-value products. In addition, trash from other businesses and cities can serve as sources for the extraction of nanocellulose. In a separate investigation, nanocellulose crystals were recovered from discarded cigarette filters using ethanol extraction, bleaching, alkaline treatment, and acid hydrolysis. The released nanocellulose crystals had breadth, an average length, and crystallinity of 8 nm, 143 nm, and 96.77%, respectively. High-purity nanocellulose may have uses in the realm of biomedical chemicals. Using autoclave, high-pressure, and high-shear homogenization, nanofibers of cellulose were recovered from lime residues. Cellulose nano fibers having a diameter of 28 nm and a crystallinity of 44 to 46%, showing water replication, and a potential utilization in the packaging industry.

Cellulose

The most abundant substance in nature is cellulose, a non-combustible green substance produced by green plants from water, carbon dioxide, and sunlight. Cellulose can be considered a biodegradable and renewable source of energy, as well as an immature material for the synthesis of other compounds, because to its simple yet adaptable chemical structure, which can yield a wide range of active polymers fibers, and films. Cellulose's physical robustness is attributed to the fact that it is often a shiny polymer. Cellulose is a key component of biodegradable waste, and its effective utilization in the form of value addition may provide a glimpse into the promising future of biodegradable waste utilization.

Cellulose is a ringed glucose molecule having a linear chain with conformations resembling flat bands. It has a repeating unit (Fig. 1) composed of two anhydrous-glucose rings ($C_6H_{10}O_5$)_n, where n is between 10,000 and 15,000. n is dependent on the material source of cellulose coupled via covalent connections of oxygen to C1 of one glucose ring and C4 of the neighboring ring (1,4 linkages) and so-called 1,4 glucosidic bonds. The hydrogen interactions between oxygen and the hydroxyl groups of nearby ring molecules stabilize the bonds and give the cellulose chains a linear structure. This molecular arrangement encourages the parallel arrangement of the double cellulose chains that comprise the basic fibrils, which then aggregate into bigger microfibrils with a diameter of 5–50 nm and a length of several micron meters. The network of inter- and intra-chain hydrogen bonding renders cellulose fibrils a moderately stable polymer with strong axial stiffness. In the cellulose chain, the cellulose fibrils contain two parts, such as a highly organized (crystalline) structure and irregular (amorphous) regions. It serves as the fundamental phase of tissue reinforcement in plants, trees, marine organisms (Tunicata), algae, and bacteria (certain bacteria release cellulose fibrils that form exterior tissue structures) (Moon et al. 2011).

About 40 to 50% of wood is cellulose, while over 90% of cotton is cellulose (Hsieh 2006). Cellulose derived from wood fiber has numerous contaminants that can be removed by dissolving it in a solution of sodium hydroxide and carbon disulfide (CS₂). This dissolution procedure will produce a viscous liquid. When the liquid is introduced into the pipe in the acid bath, rayon fiber is produced. Cellophane paper is the result of a similar method for producing thin films from cellulose. Other forms of cellulose are referred to as Microcrystalline Cellulose (MCC), Nanocrystalline Cellulose (NCCs), Nanohiskers, BC, etc., based on their properties

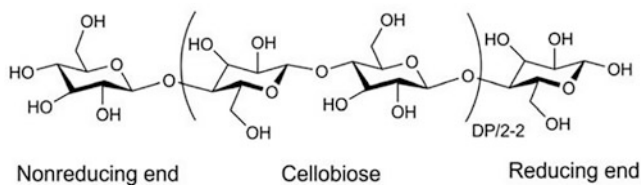


Fig. 1 Cellulose chain repeating structure

and production procedure (Diez-Pascual and Diez-Vicente 2014). Cellulose and its derivatives may also be processed into various forms, such as BC, which employs important microorganisms in the manufacture of cellulose. Under moist conditions, the modulus of elasticity of homopolymer cellulose is around 134 GPa in the axial direction. Matrix polymers such as hemicellulose and lignin have an elastic modulus of 40 MPa and 2 GPa, respectively. The interaction between plant matrix polymers and rigid cellulose fibrils in the cell walls is one of the most difficult aspects of plant mechanical performance to comprehend (Pandey et al. 2010). The main sources of nanocellulose are plants, animals, and microbes. The extraction of nanocellulose from various biomass sources for usage in many fields has garnered increasing attention.

Nanocellulose includes mostly three categories: cellulose nanocrystals, also known as nano crystalline cellulose, nanocellulose whiskers, and cellulose microcrystals that are highly generated by acid hydrolysis from lignocellulose. Amorphous cellulose also leaves nanocrystalline cellulose behind. Mechanically, chemically, or enzymatically, cellulose nano fibers, as well as other microfibrillated cellulose, nanofibrils, and microfibrils, are treated. Defibrillation treatment of cellulosic fibers and bacterial cellulose, also known as bacterial nanocellulose, microbial cellulose, and biocellulose, is the major constituent of numerous *Acetobacteraceae* species. As a biodegradable and biodegradable substance, nanocellulose can be derived from the biomass of many plants, bacteria, algae, and other invertebrates by a variety of processes. Nanocellulose shares the inherent chemical structure of cellulose with a variety of hydroxy groups, aldehyde groups, and carboxy groups to maintain its activity. Due to nanocellulose's structure, it possesses a high surface area, crystallinity, mechanical strength, and active functional groups, making it applicable in a variety of situations. The nanocomposite uses of cellulose nano fibers, also known as microfibrillated cellulose, cellulose microfibril, and microfibril cellulose, are currently of interest to researchers. To manufacture these structures, cellulosic fiber must be subjected to mechanical dispersion to cause fiber delamination, thereby separating the nanometric fiber. This procedure has a significant energy requirement to ensure the efficient operation of the nanofibrillation process. Various treatments have been devised to integrate cellulose fiber prior to its nanofibrillation to reduce and enhance the process efficiency. There are a number of methods for obtaining glucose monosaccharides from cellulose. One of the most potential applications of cellulose, which degrades cellulose polymer into smaller polymer branches, particularly glucose and cellobiose dimer.

Enzymatic hydrolysis is a less advanced approach for creating nanocellulose than acid or alkaline procedures. However, this approach has some useful characteristics, so it is vital to develop and invest in enzymatic production methods. These enzymes are traditionally classified into three groups: cellobiohydrolases, exoglucanases, and endoglucanases. The resulting cellulose nanofibers are more homogeneous than those obtained solely through mechanical treatment. Due to a chemical interaction between cellulose and monochloroacetic acid in the presence of sodium hydroxide, the hydroxyl cellulose series is replaced by carboxymethyl groups during the carboxymethylation phase, which occurs in two stages. Carboxymethylation

generates a highly charged region around the fibrils, which aids their release during decontamination treatment.

Catalytic oxidation was carried out using 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) through the incorporation of the use of a selective oxidative catalyst that could negatively charge the fiber surface, resulting in electrostatic expulsion of anionic carboxyl groups between oxidized fibers, in order to bypass the binding forces. It is a widely used and effective treatment that reduces energy consumption by up to 100 times during the mechanical dispersion process and results in the fabrication of highly uniform cellulose nanofibers between 3–4 nm and several microns in length; nevertheless, it is expensive. The creation of nanofiber structure was facilitated by the TEMPO reagent. When this technique is paired with the action of sonication, cellulose is degraded significantly, resulting in fibers characterized by prolonged separation and facilitating stages of the defibering process. It was also discovered that the employment of the TEMPO agent in the treatment of oxidized cellulose pulp results in an increase in the level of polymerization, with the nanocellulose producing a light intensity as a result of post-oxidation.

Cellulose Feedstock

Primary natural cellulose sources include:

Plants

Plants are the primary potential source of cellulose since they are abundantly available and simple to harvest. The plant's cell wall is composed of stiff cellulose and non-cellulosic matrix polymers. The production and arrangement of cellulose microfibrils serve a crucial role in sustaining the physiological functions and stiffness of the cell walls during cell growth and development. Cellulose microfibrils production refers to the manufacturing of microfibrils from cellulose source plant materials such as sisal, cotton, flax, hemp, tubers, wheat straw, sugar beets, and so on. The cellulose microfibril of plants has a cross-sectional dimension of approximately 2–3 nm (Song et al. 2020). Wood is the primary source of cellulose fibrils, although cellulose microfibrils are strengthened by amorphous intracellular materials such as hemicellulose, lignin, pectin, etc. In order to extract pure cellulose microfibrils from lignocellulosic fibers, chemical processes such as alkalization, bleaching, and acid hydrolysis are required.

Tunicate

Tunicates are a family of marine animals known as “sea squirts” (Ascidacea). There are around 2300 species of this filter-feeding marine invertebrate species. These animals are an excellent source of cellulose microfibrils due to their thick, leathery

skin. There are several species in nature, and cellulose is extracted in diverse ways depending on the species. The properties and structures of the cellulose microfibrils of the tunicates species were not comparable; nonetheless, modest changes in the synthesis of cellulose microfibrils could alter microfibril properties (Thakur and Thakur 2015). It is anticipated that the Tunicate structure and characteristics of cellulose microfibrils are comparable amongst species. Due to the varied production of cellulose microfibrils, there may be slight changes in the structure of the resultant microfibrils. *Metandrocarpa uedai*, *Halocynthia papillosa*, and *Halocynthia roretzi* are the species that have been researched the most frequently (Pandey et al. 2010). Future isolation on a commercial scale of cellulose nanocrystals with a high aspect ratio (i.e., length/width) is most likely to be possible from tunicates, among the various natural cellulose sources. In the past, the lack of tunicates, the difficulty of harvesting tunicates on a large scale, and the paucity of information on cellulose separation were the greatest obstacles to increasing cellulose isolation.

Algae

The cell wall in cellulose nanofibrils is produced by algae. Algae produce microfibrils through the self-assembly of cellulose chains, which eventually aggregate into cellulose fibers, bigger assemblies. This cellulose fiber has sections of both irregular (amorphous) and regular (crystallite) structures. Their extracellular layers of algal taxa, such as the green algae/Chlorophyta (e.g., Chlorophyceae, Ulvophyceae, Trebouxiophyceae) and the red algae/Rhodophyta or Charophyta, are claimed to include hemicellulose and cellulose (e.g., Charophyceae). Within the Ochrophyta phylum, brown algae (class Phaeophyceae) are commonly known to contain hemicellulose and cellulose (e.g., xylofucoglucan sulfate), whereas a small number of species of golden algae (class Chrysophyceae) and yellow-green algae (class Xanthophyceae) have cellulose as a structural polysaccharide. Certain dinophytes (thecate dinoflagellates) contain a protective cellulose plate (theca) as a complex extracellular layer known as the amphiesma. Other polysaccharide matrices, such as ulvan, pectin, agar, alginate, and carrageenan, have been identified in the cell walls of brown, green, and red algae and may be ideal algal cellulose products for enhancing their economic viability (Zanchetta et al. 2021).

Bacteria

Certain bacteria, such as *G. xylinus*, which are prevalent contaminants in acid fermentation, create cellulose by exploiting a variety of carbon and nitrogen sources. Under optimal cultivation conditions, these bacteria generate dense pellets of cellulose microfibrils. BC is distinct from cellulose derived from other sources due to the fact that culture conditions can alter the microfibril formation process. In a bioreactor, cellulose microfibrils are secreted by bacteria at several stages of biosynthesis, including cellulose cultivation and pellicle management. Temperature, nutrients,

bacterial strain, pH, agitation rate, etc., can affect polymer network development and microfibril morphology during fermentation. Changing the shape of the bioreactor can also alter the pellicle form of cellulose-producing bacteria. Pellicle management refers to the chemical and mechanical procedures used to remove contaminants from pellicle. Alkaline sodium hydroxide, potassium hydroxide, and other solutions hydrolyze and eliminate contaminants from the pellicle. The mechanical defibrillation of cellulose tissue yields microfibrils that are pure (Thakur and Thakur 2015).

Bacterial Nanocellulose

The bacterium nanocellulose is one of the most specialized nanocellulose species, relative to other species, due to its creation via combining sugars for low molecular weight operations. During the separation of cellulose nanocrystals and nanofibers from lignocellulosic biomass, this nanocellulose is reconstituted with its own cellulose fiber package. The features of bacterial nanocellulose include great purity, including the structure of the nanofiber network, high water content of 99% and heat-stable hydrogel bodies produced by mechanical means. Due to the single filaments' thickness of ten nanometers, scanning electron microscopy is ideally suited for examining the morphological characteristics of bacterial nanocellulose. The primary benefit of this method is that it does not necessitate the restoration of extremely small particles, as is required for transmission electron microscopy. In addition, its great depth is incredibly useful for visualizing bacterial components with three-dimensional micro cellulose.

Although bacterial nanocellulose has the same chemical formula as cellulose isolated from plants, its features are unique in comparison to those of other nanocellulose species. Due to its biological compatibility, non-toxicity, biofunctionality, and simplicity of sterilizing, nanocellulose bacteria can give a biomaterial effect on the development of mechanical properties, depending on the system's intended purpose. The requirement for high-quality nanocellulose bacteria has prompted study into biotechnological strategies spanning from volatile to non-volatile pathways, employing techniques ranging from collection or grazing to sustainable cultural practices. These can include aerated fermenters, bubble columns, and rotating disc reactors in terms of materials. Depending on the intended application, the choice of growth method is crucial since the production method influences the structure and structure of the body and the cellulose machinery.

Therefore, the production of a bioprocess is determined by the employment of a certain type of bacteria, the construction of a traditional habitat, the concentration of dissolved oxygen, pH, and the temperature of the culture medium. The culture medium appears to be the most significant factor, as it has a significant impact on the overall cost of bacterial nanocellulose production; therefore, it is essential to identify a low-cost culture that can increase bacterial nanocellulose yields in order to define a commercially viable industrial-oriented solution applicable to numerous industries. The remarkable features of the bacterial nanocellulose material make it highly adaptable and functional; hence, it is anticipated that tissue engineering and

regenerative medicine will experience major advancements in the future. However, the greatest obstacles to using cellulose bacteria are its pricey manufacture, high media expenses, and limited industrial output. As previously stated, the use of bacterial nanocellulose in medical devices is expected to result in significant advancements in a variety of medical fields; however, this should not be done in the conventional manner; rather, due to the intense competition in the field, each application must be reviewed individually.

Bacterial Cellulose Biosynthesis

BC was discovered in 1886 by Adrien Brown, who observed the production of a white, gelatinous substance on the surface of a liquid medium, which had the ability to expand to a thickness of 25 millimetres and proved to be robust when he researched acetate fermentation. Brown further confirmed that the membrane of the bacteria was originally referred to as *Acetobacter xylinum*, but was later categorized as *A. xylinum* and is now known as *G. xylinus*. Multiple bacterial and cyanobacterial species have been identified as BC producers. *Acetobacter*, *Agrobacterium*, *Gluconacetobacter*, *Rhizobium*, *Pseudomonas*, *Alcaligenes*, and *Sarina* are cellulose-producing bacteria strains (Zou et al. 2017). The most effective producer is *G. xylinus*, a species known for its ability to synthesize cellulose commercially. Plant and bacterial cellulose have comparable chemical compositions, but their physical qualities and fiber structures are distinct. Plant cellulose consists primarily of cellulose I structure, whereas BC is thought to have distinct I and II crystalline forms (Torres et al. 2012). The distinctive features of BC include a high degree of polymerization, tensile strength, crystallinity, water-holding capacity in the range of 60 to 700 of dry weight, and an ultra-fine network nanofiber structure with a diameter of less than 19 nm (Chang et al. 2012). Further study revealed that cellulosic materials had the same chemical composition as plant-derived cellulose, and BC remains the purest form of natural cellulose to date (Brown 1886).

BC and plant cellulose are both constituted of polysaccharides including α -1,4-D linear chains of glucopyranose residues that vary in purity, degree of polymerization, and crystallinity (Yan et al. 2017). The benefits of BC fiber include simple manufacture and application (Esa et al. 2014; Tang et al. 2010), very high purity, and the absence of hemicellulose and lignin found in plant cellulose. It is considered a suitable material for the future of biomaterials. Conventional BC has an elastic modulus of 3.0 GPa and a strength of 70 MPa (Rahman and Netravali 2017). They consist of cellulose nanofibers with a network of interconnected fibrils that contain linkages and aggregation and form a three-dimensional structure (Suryanto 2017).

The bacterial production of cellulose is a multistep process including regulatory proteins, catalytic complexes, and specific enzymes. It has four enzymatic processes including glucose as the carbon source (Fig. 1). The enzymatic process consists of (i) glucokinase enzyme process for glucose phosphorylation, (ii) phosphoglucumutase enzyme process for isomerization of glucose - 6 - phosphate

(Glc-6-P) into glucose - 1 - phosphate (Glc-1-P), (iii) UDPG-pyrophosphorylase (UGPase) enzy UDPGlc is the immediate precursor to cellulose in many species. UG Pase is believed to play a crucial part in the manufacture of cellulose, as this enzyme is 100 times more active in cellulose-generating bacteria than in non-cellulose producing bacteria. When disaccharides, i.e., maltose and sucrose, fed into cellulose-BC biosynthesis, which produces bacteria as a carbon source, begins with the hydrolysis of disaccharides into glucose and fructose (monosaccharides) (Lee et al. 2014).

Gluconoacetobacter xylinus (*G. xylinus*) biosynthesis of cellulose begins with the creation of individual (14) chains between the outer membrane and plasma of the bacterial cell. One *G. xylinus* cell polymerizes up to 200,000 glucose molecules with (1,4) glucan chains, which are then discharged from the cell's surface pores (Thakur and Thakur 2015). The BC chains are subsequently interconnected to form protofibrils with a diameter of around 2–4 nm, which are eventually aggregated into microfibrils that are 3–15 nm thick and 70–80 nm wide (Helenius et al. 2006). Microfibrils essentially extend into crystalline cellulose bands, the interweaving of which produces a pellicle (Fig. 2a) containing a network of BC fibrous networks (Fig. 2b). It is unknown why these bacteria produce cellulose, but the mechanisms by which they maintain life are close to the surface of the fermentation medium because oxygen content is high close to the surface, preventing the entry of enemies and heavy metal ions into the cellulose culture and providing a protective layer against ultraviolet radiation. Moreover, the nutrients are easily digestible as bacterium meal (Thakur and Thakur 2015).

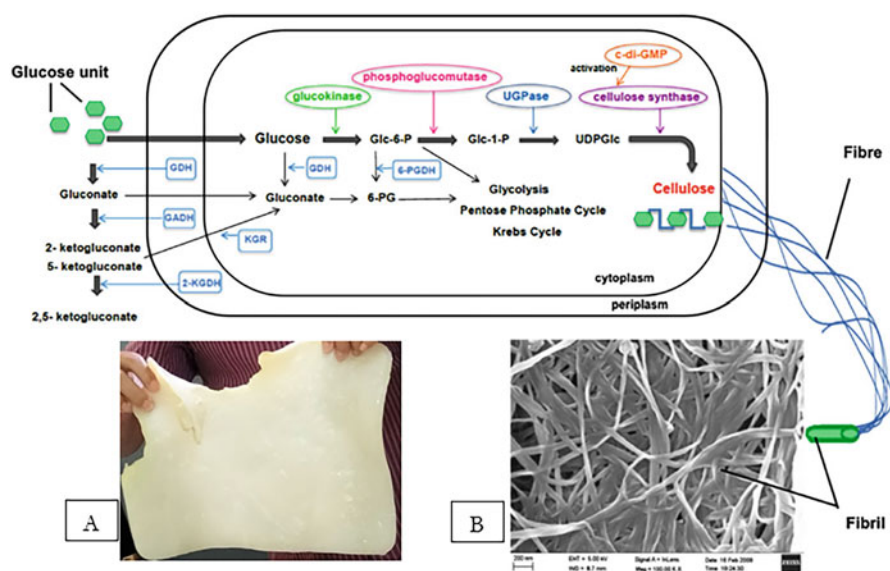


Fig. 2 Biosynthesis of BC of Bacteria. (a) Photograph of BC pellicle, and (b) Scanning electron microscopy of BC. (Adapted from Lee et al. (2014))

Production of Bacterial Cellulose from Agro-Industrial Waste

Utilizing industrial waste in production in British Columbia has numerous economic benefits. It can lower the prices of raw materials, waste disposal, and waste management for industrial applications on a big scale. Environmental accumulation issues are usually related to garbage accumulation. In order to obtain goods with additional value, the usage of these raw materials is geared toward environmentally responsible procedures. Agro-industrial wastes can be solids, slurries, or liquids, all of which are potential sources of environmental pollution. They come from cereals (barley, sorghum, corn, wheat, and rice), jute, groundnut, cotton, legumes (tomato, bean, soy), tea, chocolate, coffee, fruits (coco, mango, banana, apple), and palm oil. This waste is formed of cellulose compounds containing essential oils and fatty acids and lignocellulosic biomass including protein, carbohydrates, and nitrogen, depending on the type and method of agricultural labor. In the past decade, as shown in Table 1, numerous studies have employed agro-industrial wastes to create BC.

Producing BC nata or pellicle from agro-industrial particularly pineapple peel extract was accomplished by pulverizing clean pineapple peel in a blender (Fig. 2a) and then straining pineapple peel juice (Fig. 2b). The extract is next sterilized by boiling and placed in a fermenting pan (Fig. 2c and d). The nata was inoculated with bacteria, sealed, and fermented for 14 days. The nata is collected, cleaned, and used for other purposes after 14 days. The clean pellicle was refined with a 1% NaOH solution for 24 h to produce nanocellulose from BC pellicle. After being washed, the pellicle was sliced into little pieces to aid disintegration with a high-speed blender. Five minutes were spent alternating 50 g of pellicle with 100 ml of distilled water in a blender. After 3 min of mashing, add 1 l of water and continue mashing for an additional 2 min to obtain a satisfactory cellulose solution. Using High-Pressure Homogenizer (HPH) equipment, the homogenization procedure was carried out at 150 bar pressure five times for optimal results. The BNC slurries were then filtered to minimize their water content, poured into molds, and dried to produce a membrane or foam.

The HPH procedure data were then filtered for further use in calculating the BNC concentration in the nata solution (Fig. 3). Determine the concentration of the nanocellulose by placing it in distilled water and swirling it with a magnetic stirrer until it is uniform. The homogenization process was aided by an ultrasonic homogenizer. The homogenization products were poured into a flat mold and dried for 24 h at 70 °C in a drying oven or in a vacuum freeze dryer to produce a membrane or foam respectively.

The carbon source is the most expensive component of the cellulose synthesis process. It can account for up to 65% of the total cost of biotechnological processes (R. A. M. de S. Andrade et al. 2015). The yield of BNC synthesis is highly dependent on the medium components and the carbon source. Numerous scientists employ inexpensive waste materials as substrates in fermentation media to reduce the cellulose production cost. Furthermore, the use of waste

Table 1 Composition of fruit-industrial wastes

Fruit-industrial waste	Chemical composition (% w/w)							References	
	Cellulose	Hemi-cellulose	Lignin	Carbohydrate	Ash	Total solids	Moisture	Total carbo (%)	Total nitrogen
Potato peel waste	2.2	5.5	4.7	8.7–12.4	7.7	–	83.3–85.1	1.3	2.1
Orange peel	69.096	5.433	19.80	86.38	0.052	–	73.53	0.687	1.10
Coffee skin	23.77	16.68	28.58	44–50	5.36	–	9.17	14.41	12.9
Pineapple peel	18.11	5.2–23.2	1.37	56.13–74.08	6.80–7.58	93.6	91	40.8	0.99
Banana peel	16.62	7.34	4.01	53.01	4.04	–	5.16	–	–
Cashew apple residue	57.7	9.1	24.3	76.68	1.42	–	8.82	–	–

(continued)

(Joshi et al. 2020; Hijosa-Valsero et al. 2018; Hossain et al. 2018)

(Jiménez-Castro et al. 2020; Ayala et al. 2021; Babiker et al. 2013)

(Clemente et al. 2015; Muzaifa et al. 2021; Thaiphanit et al. 2020)

(Aruna 2019; Dahunsi et al. 2022)

(Singanusong et al. 2013; Xie et al. 2016)

(R. A. M. de S. Andrade et al. 2015; de

Table 1 (continued)

Fruit-industrial waste	Chemical composition (% w/w)								References
	Cellulose	Hemi-cellulose	Lignin	Carbohydrate	Ash	Total solids	Moisture	Total carbo (%)	Total nitrogen
									Araujo Padilha et al. 2020)
Coconut husk	23.25–55.55	12.25–14.95	22.30–38.80	21.99–22.30	0.62–1.53	86.2	–	–	–
									(Din et al. 2021; Arisht et al. 2019; Karouw et al. 2020)
Oil palm pulp	23.7–65	20.6–33.5	14.1–30.5	4.41–15.12	0.53	1.49	55–65	–	–
									(Yin et al. 2019; Boadu et al. 2021)
Dragon fruit foliage	30.8	27.5	14.4	8.5–13	11.8	–	80	–	–
									(Urün et al. 2022)
Papaya skin	20.4	24.6	2.7	20.04–30.35	3.15–5.25	10–12	54.48–68.39	38.10	1.49
									(Hamid and Zulkiffi 2021; Pathak et al. 2019)

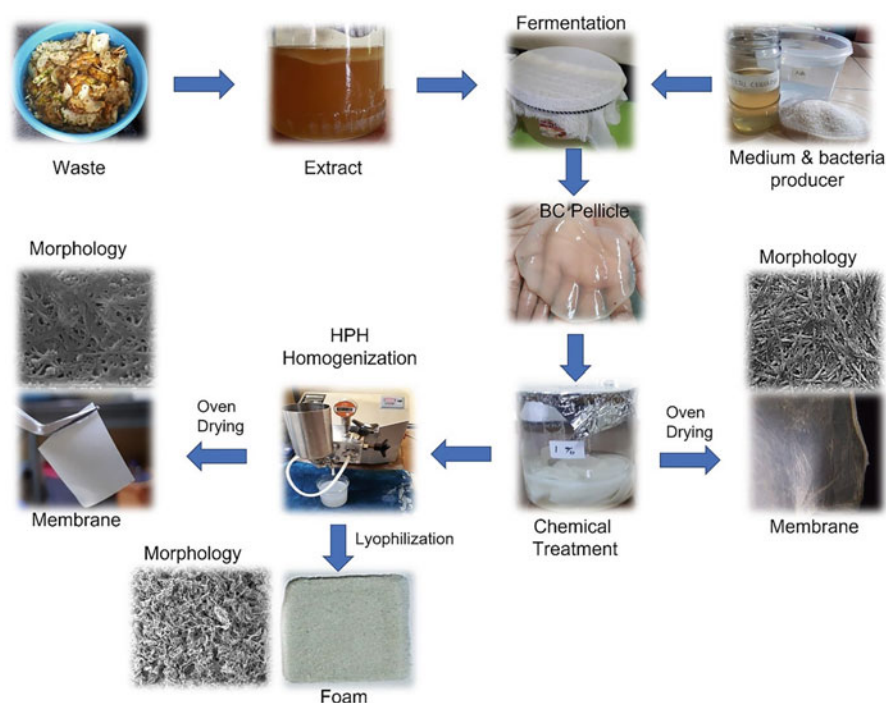


Fig. 3 Production of BC and processing methods

materials in fermentation media increased the efficiency of BNC biosynthesis compared to the use of monosaccharides as carbon sources (Wu and Liu 2012).

Numerous agro-industrial wastes, including potato peel waste, orange peel, coffee skin, pineapple peel, banana peel, cashew apple residue, coconut husk, dragon fruit foliage, and papaya skin, have been explored for the generation of BC. However, there is still much interest in discovering and employing inexpensive substrates that could increase the yield of BNC. In several instances, the culture media supplement, including extracted plant waste components, altered the structure of cellulose. The tensile strength of cellulose formed from rice husk-based medium, which contained hemicelluloses, lignin, and mineral salts, was 79% greater than that of cellulose derived from normal glucose-based medium or reference BC (Goelzer et al. 2009). In addition, the use of biomass waste in the synthesis of BC has positive environmental effects.

The majority of BC products use fruit extracts and coconut water (Suryanto et al. 2020). In 2020, Indonesia was the third largest exporter of processed pineapple in Southeast Asia, behind Thailand and the Philippines, producing approximately 2.45 million tonnes per year (BPS 2021). Supported by a suitable tropical climate, nearly all of Indonesia's area is devoted to pineapple production, resulting in a substantial volume of pineapple peel waste. Sugar, protein, organic acids, nitrogen, minerals,

water, and bromelain are all components of pineapple. It also contains trace levels of every vitamins, except vitamin D. Additionally, pineapple peel is frequently processed into syrup and extracted liquid for animal feed. Bacteria could potentially use pineapple peel as a source of nitrogen and carbon to produce cellulose.

Fermentation Methods

There are two distinct techniques of fermentation for producing cellulose, namely dynamic and static fermentation. In a dynamic fermentation with agitation procedure, BC pellicle is formed into pellets whose size and shape depend on the fermentation and agitation pace. In this instance, pellicle exhibit a greater water-retention capacity, reduced crystallinity, and a disordered structure (Kosseva et al. 2020; Algar et al. 2015). In static fermentation, the liquid/air interface of the liquid nutrient solution produces pellicle. Figure 2a demonstrates that, depending on the container used, multiple pellicle shapes can be created in the form of a film. The thickness of the pellicle is dependent on the fermentation period, which typically does not exceed 14 days, as inhibitory metabolites like as formic and glycolic acid accumulate during prolonged fermentation (Erbas Kiziltas et al. 2015). In this fermentation process, bacteria often form parallel layers of cellulose with a strong and rigid structure (Urbina et al. 2021).

Depending on the bacterial strain, oxygen, carbon sources (often saccharides), temperature, and pH must be added to the fermentation medium. Typically, fermentation occurs at a pH range of 4–7 and a temperature range of 28–30 °C. pH mediums below 4 are not conducive to bacterial development (Sadh et al. 2018). However, a newly identified strain of bacteria (*Gluconacetobacter medellensis*) is able to grow at an extremely acidic pH of 3.5 to manufacture BC with superior characteristics and in large quantities (Castro et al. 2011). This is advantageous in the fermentation process since most microorganisms cannot survive in low pH media, hence preventing contamination with bacteria, fungus, and other yeasts, particularly in large-scale manufacturing.

H–S media, which was created by Hestrin and Schramm, is the most often utilized medium for the production of BC. Citric acid, dibasic sodium phosphate, yeast extract, peptone, and D-glucose comprised the standard medium (Hestrin and Schramm 1954). Various carbon sources, such as sucrose, fructose, mannitol, and glycerol, among others, have been used to modify these compositions (Mohammadkazemi et al. 2015). However, when commercial nutrient sources are used as carbon additions, they increase production costs. Especially for industrial-scale production and BC applications, this is not economically practical. Consequently, a number of researchers have concentrated to develop the cost-effective carbon feedstocks derived from residues and byproducts of various industrial activities as well as agrowaste for the synthesis of BC.

There were multiple attempts to optimize the culture conditions, medium composition, and scaling-up procedure. BC is often synthesized on Hestrin-Schramm (HS) media, which contains glucose, peptone extract, and yeast as carbon and nitrogen sources, respectively. However, glucose is an expensive carbon source for the manufacture of BC. It results in the development of byproducts such as gluconic acid, which lowers the pH of the culture medium and, thus, reduces BC synthesis (Thakur and Thakur 2015).

Several industrial wastes, such as tea infusion, wheat fatty acid hydrolyzate (Hong et al. 2011), grape bagasse and raw glycerol, beet molasses, sugar cane, and corn steep liquor, Konjac powder, fruit juices, including oranges, Japanese pears, apples, pineapples, and grapes, grape juice extracts and sulfur berry liquor, and dried olive plant residues, have been investigated as potential sources of BC. Developing cultivating media based on less expensive raw ingredients will reduce the cost of BC production.

Increasing BC production by adding various chemicals to culture media. Alcohol, vitamin C, lignosulfonates, water-soluble polysaccharides, and thin stillage from wine distillation are examples of chemicals. Six alcohols were administered at varying concentrations during the static fermentation of *G. xylinus* cells (Lu et al. 2011) in order to determine the stimulant perspective of alcohol. All alcohols examined boosted BC production, with n-butanol being superior to ethylene glycol, glycerol, mannitol, methanol, and n-propanol. However, the results demonstrated that n-butanol increased BC synthesis only when added at concentrations below 1.5% v/v (maximum production of 132.60 mg per 100 ml, 56.00% higher than control). In contrast, mannitols only stimulated the bacteria to manufacture BC at a maximum concentration of 4% v/v (maximum production of 125.20 mg per 100 ml, 47.30% higher than control) (Thakur and Thakur 2015).

Gluconacetobacter, Sarcina, and Agrobacterium bacterial species have also produced cellulose. However, only Gluconacetobacter species produce commercial quantities of cellulose. *G. xylinus* continues to be utilized in cellulose synthesis and research. It is a nonpathogenic rod capable of synthesizing relatively large quantities of cellulose from carbon and nitrogen sources. The *G. xylinus* bacteria occurs naturally in unpasteurized fruit, juices, beer, and wine due to sugar fermentation. Recently, the generation of BC from a *G. sacchari* strain employing a carbon source yielded equivalent results to those obtained with *G. xylinus*. Due to their abundance, renewability, biocompatibility, biodegradability, and unique properties, the usage of BC has garnered substantial global attention over the past decade in an effort to raise awareness and demand for biobased eco-friendly functional materials (especially nanometric dimensions and nanostructured networks). The synthesis of nanocomposites with a variety of mixes, including synthetic polymers, natural bioactive substances, and inorganic compounds, is the subject of intensive research and development by British Columbia.

Bacterial Nanocellulose Fibrillation Using High-Pressure Homogenizer (HPH)

The first time Herrick and Turbak used this technique to separate Nano Fiber Cellulose (NFC) from wood fibers was in 1983. Homogenization is one of the mechanical processes involved in the manufacture of NFC. In addition to homogenization, reeving, cryo-crushing, milling, and microfluidization were utilized. A homogenizer-like mechanical use for isolation might also be considered another mechanical technique. Refining, cryo-crushing, and grinding are typically employed in conjunction with homogenization to lower the particle size from micro to nano. Pressure, varying transit periods through the engine (cycles), suspension concentration, and temperature (Kawee et al. 2018) are crucial elements that affect the characteristics of the produced nanofibers. The HPH application successfully enhances cellulose fiber, simplicity, efficiency, reproducibility, industrial scalability, and organic usage without chemical solvents (Li et al. 2012; Wang et al. 2015). However, homogenization is a simple process that does not require chemical solvents. Homogenization is not devoid of issues, but clogging is one of the most essential issues connected with non-uniform micro-sized particles in the early phases, leading to channel blockage. To overcome this issue, an early stage of treatment is required, including the use of pretreatments such as refining, cryo-crushing, and milling to lower the uniform fiber size to below 100 nm.

The high-pressure homogenizer is a device used to reduce and homogenize the particle size of both solids and liquids in solution. HPH is typically employed in the preparation of pharmaceutical and cosmetic components (Abdul Khalil et al. 2014). Figure 4 depicts the process in HPH equipment (Fig. 3) in which a solid particle combined with a solution is sucked into the cavity and subsequently pushed through a narrow gap by a pressure piston, as shown in Fig. 4b. The composite particles undergo dramatic variations in velocity in the material flow, known as cavitation force. Shear force refers to the fragmentation (crushing) of particles as a result of

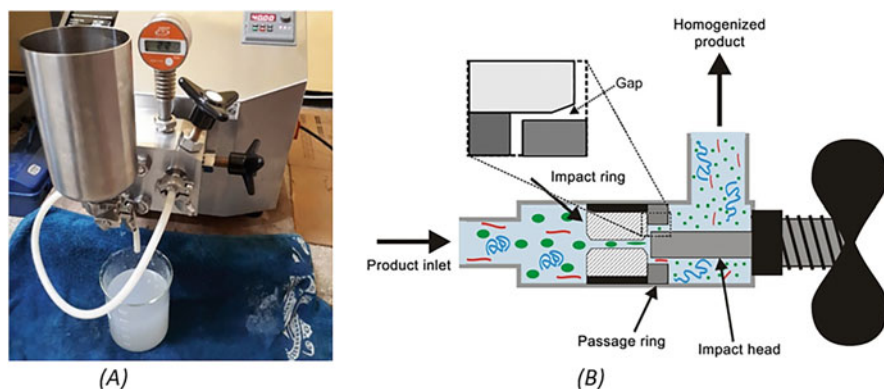


Fig. 4 (a) High-Pressure Homogenizer (HPH) equipment; (b) HPH working scheme (Augusto et al. 2017)

their high-velocity collision with the hollow or narrow slit wall. In addition, cavitation force and shear force contribute to the high velocity of particle impact between particles. The fragmentation of particles into smaller sizes produces a homogenized product (Kawee et al. 2018).

The utilized mechanism exerts high pressure on the solution and goes via a small opening in the pressure-regulating lever. In the homogenization process, HPH generated a combination of shear, impact, and cavitation forces based on precise system settings, including pressure settings and repetition (cycles).

Engineered Bacterial Nanocellulose

BC can be utilized in several forms, such as intact membranes (or pellets or fibers, depending on the production procedure), BC nanocrystals (BCNC), and deconstructed BC. BC was created to obtain particular qualities to fulfill the product's property requirements. Engineering of BC is accomplished by modifying BC using surface functionalization, functional material addition, nanoparticle combination, and polymer composites. Among the techniques for modifying BC are impregnation, disassembly, acid hydrolysis, and in-situ process (Azeredo et al. 2019). Different procedures involving the chemistry of the hydroxyl group can be used to functionalize surfaces (e.g., etherification, esterification, and oxidation). This will enable nanocellulose to have a variety of functional groups, including quaternary ammonium and aldehyde groups, which confer bacteriostatic and biocompatibility (Li et al. 2018).

BC has accomplished functional material addition utilizing an active chemical. Antiviral and antibacterial quaternary compounds (anionic and cationic) such as 4-vinyl pyridine, imidazolium, and pyridinium are added to BC to create antimicrobial agents. Aldehyde compounds are added to BC to increase its antibacterial properties for multiple uses. Oxidants like TEMPO can be used to convert hydroxyl groups to aldehyde and carboxyl functional groups.

Adding nanomaterials to BC typically has a significant effect on their function. BC has been augmented with nanomaterials like carbon nanotubes and graphene for use as electrodes for the direct electron transfer of glucose oxidase. They have numerous potential biomedical applications, including biosensors, biofuel cells, and bioelectronic devices (Kim et al. 2013). Several researchers mixed silver nanoparticles (CN101589854A), TiO₂, ZnO, and Al₂O₃ for antibacterial reasons in various filters. Copper nanoparticles in water filters (Szekeres et al. 2018) and coating materials (Zhou et al. 2020) act as antiviral/antibacterial agents. Copper surfaces can destroy viruses in as little as 8 h, whereas stainless steel and plastic surfaces require up to 72 h (van Doremalen et al. 2020).

BC is a biopolymer that has been included into composites with other polymeric substances. The majority of organic polymers have hydrogen bonding. This unique connection permits the creation of BC composites comprised of many polymers with distinct characteristics. BC composites containing biopolymers enhanced the biomedical uses, conductivity, and physicomechanical properties of BC. For instance,

the synthesis of BC-Chitosan composite was based on Chitosan's active involvement in biomedical domains as an anti-viral, anti-microbial, and anti-fungal agent, drug delivery vehicle, tissue engineering scaffold, wound healing agent, and wound exudate absorbent (Shah et al. 2013). BC-PAni composites were produced by in situ polymerization of PAni in a solution containing a BC pellicle, where PAni formed a layer on the BC surface. The composite possesses a high conductivity, which is increased by controlled acid doping (Shi et al. 2012).

Properties of Bacterial Nanocellulose Membrane

Morphology

Membrane structure is a network-like structure made of tiny fibrils that overlap and a membrane surface composed of several threads interwoven to form a layer of randomly arranged cellulose bands (Fig. 5). Ultrafine lengths of microbial cellulose ranging from 1 to 9 μm produce dense reticulate structures stabilized by hydrogen bonding. The degree of polymerization of plants ranges between 13,000 and 14,000, whereas the degree of polymerization of BC ranges between 2000 and 6000 and even reaches 20,000 (Bielecki et al. 2005).

The shape of BC is dependent by fermentation techniques and bacterial strain. Bacteria are capable of producing solid sphere-shaped BC or flock-shaped BC (Bi et al. 2014). The morphology of the BC film comprises of numerous micrometer-long cellulose nanofibers with an average diameter of approximately 70 nm. The shape of BC nanofibers is characterized by fibrils with linkages and aggregates that are interlinked. As demonstrated in Fig. 6, it generates a three-dimensional structure with variable cavity or pore diameters. These holes are acceptable for use as the composite system's matrix. However, the existence of pores can reduce the tensile strength of a membrane by trapping water molecules and weakening the structure of the fibers (Pa'e et al. 2014).

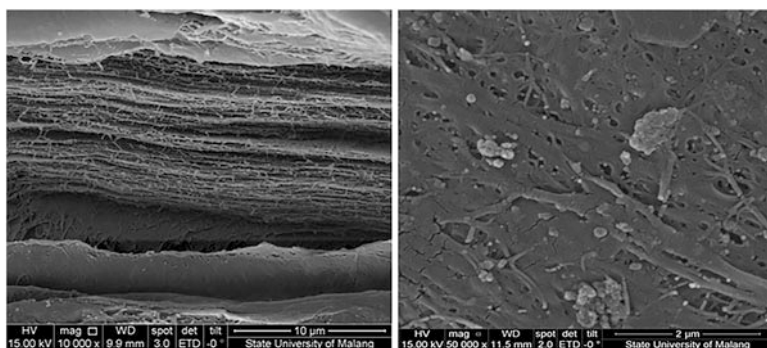


Fig. 5 Side view of BC membrane (Left) and top view of BC membrane (Right)

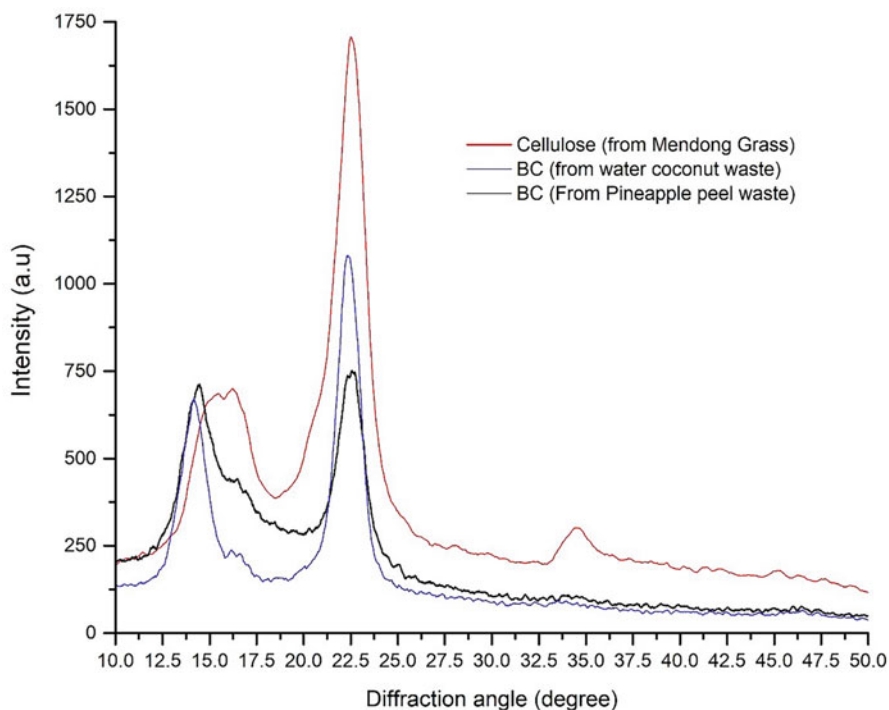


Fig. 6 Diffraction pattern of BC from agro-industry waste and the cellulose fiber from plant

Structure

Figure 6 displays the X-ray diffraction results comparing the structures of BC from waste coconut and pineapple peels with plant-derived cellulose. The bacterial cellulosic diffraction pattern has three peaks at 2θ angle: 14.20° , 16.70° , and 22.40° , but the cellulose fiber from the mendong plant has four peaks: 14.90° , 16.50° , 22.60° , and 34.50° . The first three diffraction peaks correspond to the 10, 110, and 002 crystal planes (Vicente et al. 2017), whereas the last three diffraction peaks from plant fibers correspond to the 040 crystal planes (Suryanto et al. 2018).

Diffractogram patterns can be used to determine the cellulose structure of fibers. The dip between the two peaks in the region at 2θ about 18° indicates the amorphous portion of the cellulose fiber structure, whereas the peaks between 22° and 23° are indicative of native cellulose (cellulose I) (Le Troedec et al. 2008). Therefore, cellulose I is the structure that forms bacteria from coconut water and cellulose. The two leading peaks denote an additional form of cellulose I, wherein the I cellulose structure has peak diffraction angles of 14.90° and 16.70° . In comparison, the structures of cellulose I exhibit peaks at 16.80° and 14.30° (Cheng et al. 2011). Observing the peak of the BC nanofiber diffraction angle revealed a high intensity

peak at 14.20° and a low intensity peak at 16.70° , indicating that the structure of the BC corresponds to cellulose I. The cellulose I has a triclinic structure with the following cell dimensions: $a = 0.674$ nm, $b = 0.593$ nm, $c = 1.036$ nm (chain axis), $\alpha = 117^\circ$, $\beta = 113^\circ$, $\gamma = 81^\circ$, one cellobiose residue per unit cell, a density of 1.582 cm⁻³, and a unit cell volume of 0.3395 nm³. It is known that cellulose derived from mendong fiber has a cellulose structure I with $a = 0.801$ nm, $b = 0.817$ nm, $c = 1.036$ nm (chain axis), $\alpha = 90.0^\circ$, $\beta = 97.3^\circ$, two cellobiose residues per unit cell, a density of 1.599 cm⁻³, and a unit cell volume of 0.6725 nm³ (Suryanto et al. 2014).

The conditions of a region influence the construction of BC structures. In addition to the type of bacteria (Bi et al. 2014), the culture in the reactor and the static culture determines the tendency of the static culture to make cellulose I (Zhou et al. 2007). Moreover, the drying process of BC products relies on the drying technique (Pa'e et al. 2014). The third peak of the cellulose diffraction pattern of plant fibers has a low intensity and is located at 34.5° with a low angle. This peak represents a quarter of the length of the cellobiose unit and emerges from the arrangement in the direction of the fiber. It is sensitive to the alignment of chains into fibrils. Moreover, this peak is an indication of monoclinic structure and is expected in all natural cellulose derived from plant sources (Clair et al. 2006).

The BC degree of crystalline waste pineapple peel was 83.0% and increased to 85.0% after being washed with 5% peroxide (Suryanto et al. 2018). In comparison to BC, nata de coco has a higher degree of crystallinity and crystalline index of 86.5%. In general, the degree of crystallinity of BC is between 87 and 90% (Pa'e et al. 2014). BC has superior crystalline characteristics to cellulose fiber of mendong grass, with a crystalline index and degree of crystallinity of 83.5% and 85.0%, respectively. It suggests that the BC fiber is purer and simpler to produce than plant fiber.

Functional Groups

Infra-red spectroscopy or Fourier Transform Infrared (FTIR) is used to analyze the functional group of cellulose and the characteristics of its chemical bonds. As depicted in Fig. 7, the functional group differences between bacterial and plant cellulose are minimal. Hydroxyl group stretching vibrations at 3481 – 3415 cm⁻¹, CH₂ stretching vibrations at 2902 cm⁻¹, and CH₂ stretching vibrations at 2902 cm⁻¹ dominate the distinctive absorption peak of cellulose. C-OH absorbed water at a frequency of 1633 – 1652 cm⁻¹, C-H bending vibration at 1444 cm⁻¹, C-O-C and C-OH stretching vibrations of the sugar ring at 1085 – 1093 cm⁻¹, and -glycosidic connection between glucose units at 890 cm⁻¹ (Shezad et al. 2010). All samples' spectra exhibited a specific band at approximately 894 cm⁻¹ and a band at 1444 cm⁻¹, thereby identifying them as type I cellulose (Kawee et al. 2018). The positions of the peaks in the FTIR spectra of all samples were not significantly changed, and the cellulose type did not change (type I).

In the process of creating BNC membrane, treatments such as sonication alter the FTIR pattern. For instance, the FTIR signals of sonicated and non-sonicated BC

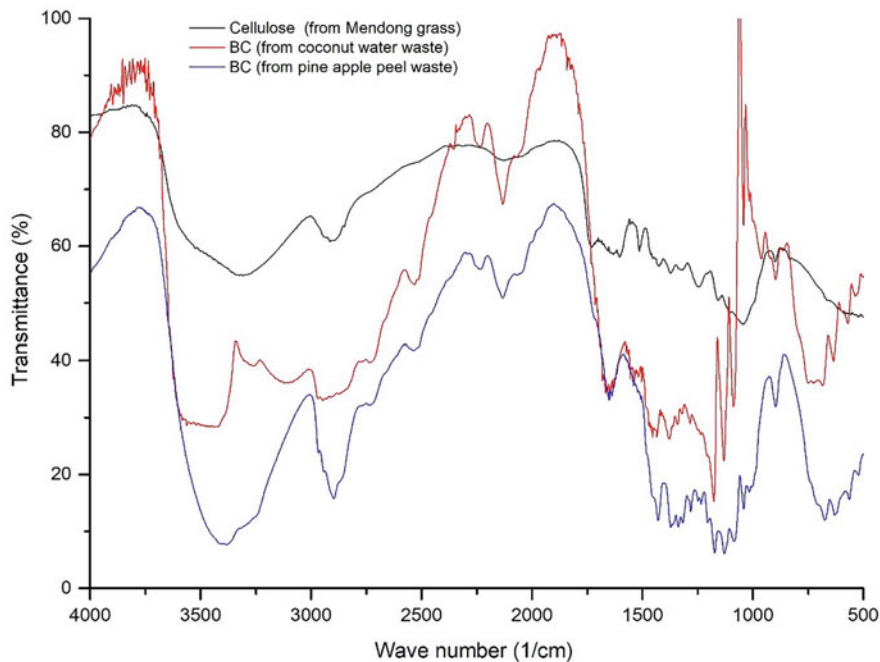


Fig. 7 Functional groups of BC and plant cellulose

were largely same, indicating that its chemical structure was not significantly altered. However, ultrasonication of variable time altered the wave number and peak intensity of the principal BC spectra. This moving principal peak illustrates the loss of the long-chain BC structure resulting from ultrasonication. The OH-band emerges at 3340 cm^{-1} with an intensity of 24% in unsonicated BC. After 90 mins of sonication, the O-H band shifted to 3335 cm^{-1} at a 21% intensity. After ultrasonication, the bands at approximately 2950 cm^{-1} and 1641 cm^{-1} , which correspond to C-H and C-O stretching, respectively, also shift. The C-H stretch for unsonicated BC emerged at 2897 cm^{-1} with an intensity of 67%, but changed to 2902 cm^{-1} with an intensity of 56% after 90 mins of sonication (Abiral et al. 2018).

Mechanical Properties

In defining the application and technological procedure for employing BC films, the quality of BC is crucial. Depending on the polymer modification technique, cellulose's tensile strength can vary. BC possesses a favorable attribute known as film strength, which influences the strength of the end goods manufactured from this polymer. BC film offers greater strength qualities than other commonly used polymers when it is in film form. Comparatively, the tensile strength of BC films (200–300 MPa) is significantly greater than that of polypropylene (30–40 MPa)

and cellophane (20–100 MPa) (Betlej et al. 2021). The strength, hygroscopicity, and water vapor permeability of BC film are significantly altered by chemical reagent modification. Additionally, nonlinear BC networks can reduce the mechanical strength of BC films (Hsieh et al. 2008). By incorporating different substances into the polymer, the desired traits and characteristics can be achieved. The addition of montmorillonite to the cellulose-based composite decreased its tensile strength while increasing the polymer's permeability. The addition of BC to paper pulp increased the paper's tensile strength and affected its stiffness (Gao et al. 2011).

Water Holding Capacity and Water Release Rate

The water holding capacity and water release rate of a nano-bacterial compound's pellicles are of enormous importance, particularly if the substance is intended for medical applications such as wound binding and tissue engineering. As a result of their high porosity and surface area per unit, bacteria nanocompounds that are hydrogels retain the majority of the water. The highest water absorption capacity of nano-bacterial compounds measured 104 g/g, whereas the lowest water holding capacity measured 97.3 g/g. These behaviors indicate that the WHC profiles are consistent with the electron microscopy scan report and confirm that agricultural industry waste has a minimal effect on the size of the fibers and holes or the fibrous branching scheme. In contrast, the water content of nano-bacterial compound products over a period of 48 h affects the rate at which water exits the films. The ability of the bacterial nanocomposite to load water is mostly attributed to the glucan-forming hydrogen bonds. The drying response of a bacterial nanocomposite occurs in two stages: surface evaporation as a visible process dependent on temperature, humidity, and air velocity, followed by heat transfer and non-bonding of interior water molecules caused by the simultaneous breakdown of hydrogen bonds.

Bacterial Nanocellulose Composite Application

The BNC's unique feature is its broad usage potential for different applications. Due to its unique physicochemical and mechanical properties, BNC can be used in various specific fields, as follows:

Biomedical Application

Blood vessels, vascular, biosensing artificial cornea, artificial bone, artificial cartilage, artificial urethra, neural tissue, adipose tissue, wound dressings, controlled cell transfection, and drug administration can all be engineered using BC. Chondrocytes are the most abundant particles that make up cartilage in the field of cartilage engineering. Since Chondrocytes do not divide, cartilage tissue repair is very slow because there are not enough vessels and nerves to carry out the necessary

metabolism; therefore, this is a significant challenge in tissue engineering. Numerous BC characteristics can be employed as scaffolds to replace cartilage, induce the development of new tissue, and promote cell proliferation and growth. Using BNC for injured bone tissue is a rapidly emerging way of bone repair, as conventional procedures like as bone grafting have their own disadvantages, such as disease transmission and immunological rejection (Nemati and Gholami 2021).

The extraordinary efficacy of BC as a root canal filling material for intracanal asepsis in experimental animals. BNC demonstrated superior biological properties and compatibility for tooth canal therapy. Comparatively, BC has a greater expansion capacity and liquid absorption capacity than conventional methods employing conventional paper point materials (de Oliveira Barud et al. 2016).

BC membranes provide potential for nanostructured drug release systems for illegal substances or active compounds such as lidocaine hydrochloride, ibuprofen, and caffeine, while also providing an effective physical barrier against external infection. BC has also been regarded as an exceptional non-allergenic biomaterial for usage in the cosmetic sector as a facial mask for dry skincare and natural facial scrubs or as a cleansing agent (Dasan 2015).

Electronics Application

The application of BC in electronics has attracted significant interest in order to build new substrates with the required thermal stability, transparency, and flexibility, as well as sustainability and recyclability, for the development of electronic devices. Nanocellulose-based nanocomposite satisfies this criterion due to the BC features of superior mechanical qualities, thermal and surface stability. Its shape makes it desirable for use in electrical applications, such as dielectric material organic light emitting diodes (OLED), solar cells, capacitors and supercapacitors, and transistors.

Dielectric materials are insulators with interfaces that contact semiconductor or conducting materials. BC possesses good intrinsic insulation properties. Therefore, its application enhances the insulating property of standard paper. BC can be used as a fractional-order material to create electronic devices from polymer fractional-order BC-based materials.

BC also introduces innovative new features to OLED manufacture. OLED devices must be transparent and flexible because they are utilized in the screens of portable devices, monitors, and televisions. When the diameter of BC is less than one-tenth of the wavelength of visible light, it becomes a medium that scatters light freely. Consequently, BC with an ultrafine network was utilized to make translucent paper. The primary benefits of electronic paper are its high reflectivity, flexibility, contrast, and biodegradability in rewritable maps, paper, dynamic wallpapers, e-newspapers, e-book tablets, study tools, biosensors or actuators, and audio speaker diaphragm membranes. BC is also superior for membranes in headphones and loudspeakers (Dasan 2015; Tsalagkas 2015).

Energy Application

Solar energy harvesting devices with good charge transport capabilities and a large surface area can capture photons and transform them into electrical energy more efficiently. The mechanical properties of BC with a three-dimensional (3D) mesoporous structure are equivalent to those of other fibrous materials (e.g., glass fiber and carbon fiber) with a very large surface area. Therefore, BC can be utilized as a template or matrix in the production of photoelectrodes via 3D nanostructure processing. In addition, hydrophilic mesopores in cellulose films may serve as optimal host matrices for embedding catalyst nanoparticles with minimal agglomeration. So, BC networks can be condensed to generate optical characteristics that optimize photon absorption (Wang et al. 2017).

The photo-electrochemical separation of water is a novel technology for converting solar energy directly into hydrogen fuel. This approach is comparable to the creation of solar cells, comprising broad-band light absorption, a wide surface area for reduction-oxidation reactions, and rapid charge formation and separation. Consequently, photo-electrochemical cells also benefit from the structural benefits provided by 3D nanocellulose-based architectures.

Most lithium-ion (LiB) battery technologies use a PVDF membrane as a binder and a volatile and toxic chemical solvent for processing, such as N-methylpyrrolidone (NMP). Both NMP and PVDF are expensive and challenging to recycle materials. The replacement of organic solvents with carboxymethyl cellulose (CMC) to prevent economic, safety, and environmental issues. CMC is a water-soluble derivative of a linear polymer of cellulose with varying degrees of anionic substitution of carboxymethyl groups. Recent research has utilized 100% cellulose paper sheets for both the separator and electrode substrate of LIBs. These cellulose paper-based LIBs are flexible and thin, which gives them an advantage in several applications where conventional LIBs cannot perform, such as batteries for flexible electronics that require irregularly shaped batteries that can conform to the shape of a space, such as in electric vehicle door trims (Wang et al. 2017).

Widely used commercial alkaline batteries has high porosity, low cost and weight, strong mechanical qualities, and excellent wettability of cellulose paper sheets. Its use in LIB is restricted due to its poor thermal shutdown performance, which halts ion flow at high temperatures. Another worry is the hydrophilicity of cellulose paper, which can lead to lithium salt deterioration by absorbing a great deal of water. Nonetheless, some researchers resolve this issue by treating cellulose paper with extended heat treatment. The cost of manufacturing this separator is projected to exceed 20% of the entire cost of the LIB. In light of the relatively low cost of cellulose, attempts are underway to employ cellulose paper and its composites as LIB separators to replace the high cost of conventional membranes (polyethylene (PE)/polypropylene (PP)), particularly for low-power applications.

Piezoelectric nanogenerators based on cellulose are another use of the mechanical energy harvesting technique. Crystalline cellulose is the most recognized polymer piezoelectric material. Until the discovery of cellulose paper (also known as EAPap, which is an abbreviation for electro-active cellulose paper) that exhibits the

phenomena of actuation, piezoelectric qualities were first identified in natural wood. Polarity of the piezoelectric arises from the alignment of glucose units along the C2 monoclinic crystalline lattice via glycosidic bonds (Kim et al. 2006).

Food Application

As a component in traditional Southeast Asian desserts originating in the Philippines, BC is known as “nata de coco.” This meal has rapidly gained popularity and expanded around the globe. BC is made by fermenting coconut water to create nata, which is then sliced into pieces and soaking in sugar syrup. BC is also utilized in the food business as a possible stabilizer, stabilization, gel, and suspending agent. For instance, ice cream containing BC can retain its form for at least 60 min following removal from the freezer. There are numerous varieties of nata, including nata de pina made with pineapple juice, nata de coco made with coconut water, nata de soya made with tofu waste, and nata made with vegetable media. BC can reduce the risk of chronic diseases including diverticulitis, obesity, diabetes, and cardiovascular disease (Shi et al. 2014; Iguchi et al. 2000). BC has been utilized to generate low-fat ice cream with enhanced stability, emulsifier, rheological, and resistant capabilities in comparison to conventional ice cream. BC can also be added to mayonnaise products as a fat replacement with acceptable rheological and sensory properties.

BC has been investigated as a packaging material in the food packaging industry due to its strong web and barrier qualities. Paper is a significant material and is viewed as a good alternative to synthetic plastics; hence, paper-based materials are selected as packaging materials due to the fact that paper is both renewable and recyclable. Utilizing nanoparticles in papermaking technology allows for the production of paper with enhanced barrier and processing, strength, and hydrophobicity features. Due to the substantial reduction in oxygen molecule penetration through densely entangled fibrous structures, the paper manufactured from BNC possesses desirable qualities such as optical transparency, thermal stability, high strength, and superior oxygen barrier capabilities. With conventional micro-sized pulp paper, this feature could not be obtained. In certain items, color film is effective for both consumer appeal and food protection from the adverse effects of light. (Urbina et al. 2021) BNC nano paper (from biomass residue) coated with poly-hydroxyalkanoates (PHAs)/biomass extract is effective for food packing.

In addition, BC has been utilized as “active packaging” with components that absorb or release substances into or from packed foods or the surrounding environment to maintain freshness, extend shelf life, or improve the conditions of packaged goods. As a wrapping for ground beef, antimicrobial Nano paper has also been utilized by impregnating BNC with lyophilized post-biotic *Lactobacillus*. Additionally, the tailored films are antibacterial against *Listeria monocytogenes*. The addition of BC to food packaging manufactured from potato skin film can increase tensile strength and decrease moisture content, oxygen permeability, and water vapor permeability.

Environmental Application

Current environmental restrictions mandate the use of bio-based products and green materials in order to invest in green processes, industrial ecology, and sustainability. In addition, efforts should be made to create a healthy aquatic ecosystem because it is well recognized that water is the end destination of direct and indirect pollution (Pawar et al. 2018). The handling of wastewater containing heavy metals is one of the technological issues associated with preserving environmental quality. BC becomes a potential alternative material for wastewater purification and reuse technology due to its robust 3D nanofibrillar network with good structure. BC satisfies the requirements to develop a particular material for environmental applications and usher in a new era of biodegradable materials.

Various industrial processes in the battery, leather tanning, and fertilizer industries are frequently linked to heavy metal contamination in the environment. Heavy metals are extremely hazardous because they have the capacity to accumulate, resulting in greater exposure of organisms to these metals than can be found in the environment alone. Heavy metals are extremely hazardous since they can accumulate in organisms after being exposed to more of these metals than can be found in the environment alone; therefore, heavy metals from wastewater must be removed from polluted rivers. The BC membrane can serve as the template for this endeavor. BNC membranes are typically paired with chelating or absorbent agents such as ethylene diamine tetra acetic acid (EDTA), graphene oxide magnetic nanoparticles, chitosan, or chemical reagent modifications such as oxidation using 2,2,6,6-tetramethylpy-peridine-1-oxy radicals (TEMPO-oxidation). BC has been demonstrated to be an effective template for removing, among other elements, arsenic (As), chromium (Cr), iron (Fe), lead (Pb), strontium (Sr), and copper (Cu), with an antimony (Sb) removal effectiveness of up to 90% (Urbina et al. 2021).

Optical Materials

Nanocellulose can be modified with different top layers, polymer chains, or other nanoparticles to achieve biotechnological sensitivity at the lowest cost; these processes enable the detection of objects with interesting features and functions on their surface, particularly through optical applications. The primary objective may be the creation of chiral and metallic compounds from cellulose nanocrystals and other polymers or silica; this enables the component to be removed selectively or utilized in nanocomposites. Special removal of the nanocomposite's original component yields a chiral mesoporous material that can handle or act as a template for other materials. To improve the material, cellulose nanocrystals were employed to add upper plasmon, UV, and fluorescence inhibitors as well as lower light reflectors.

Wood Adhesives

In wood adhesion technology, the primary adhesives are formaldehyde-based adhesives such as phenol-formaldehyde resin, urea-formaldehyde resin, and melamine-formaldehyde. However, the usage of formaldehyde is problematic due to its extracts and other organic molecules that are flexible. Because of this, bio-based nanocellulosic materials are developed to alleviate these deficiencies, promote sustainable development, and deliver the benefits of their regeneration. In addition, cellulose nanomaterials in wood adhesion formulations considerably enhance adhesive bonding effectiveness and stability under both wet and dry circumstances and function as a stabilizing agent. However, the substantial increase in viscosity generated by its addition restricts its concentration. Nanocellulose's introduction of a nano-biomaterial that is stable, bulky, and inexpensive for material development is gaining appeal among common adhesives and, in particular, wood adhesives. Improving the mechanical and physical properties of panels, changing adhesive structures, and reducing formaldehyde emissions are, thus, the primary benefits of employing nanocellulose as a means of boosting the manufacture of sustainable wood panels. The addition of cellulose nanofibers to melamine-urea-formaldehyde and urea-formaldehyde adhesives strengthened the cellulose nanofibers environment, increased strength and durability, and enhanced the mechanical properties of the board.

Cosmetics

Additionally, nanocellulose has been utilized and promoted within the cosmetics business. In recent years, cellulose microfibrils have been included in candy bags and other cosmetic goods. On the other hand, micro cellulose bacteria have a few aesthetic applications because of their unique features, particularly as a skin-supporting substance or cosmetic agent in cosmetic production. Additionally, bacterial cellulose has been utilized as a medication delivery support, which is efficient for both hydrophobic and hydrophilic medicines. Consequently, a cellulose-coated bacterial film created in the converted region was successfully used to create a sheet mask that has the effect of hydrating and treating acne- and inflammation-prone skin. There is a potential that the use of 1,3-dihydroxy-2-propanone in the formulation of microbial nanocellulose compounds will not cause skin allergies in another patient with vitiligo. This substance applied to the skin with bacterial cellulose does not produce a distinct odor, a common adverse effect of commercial cosmetics.

Textile

Due to their cellular structure and vast functional location, nanocellulosic materials have proven useful in the textile industry, particularly for medicinal purposes.

Special characteristics include non-static behavior, low pollution, and humidity levels, and superior mechanical and liquid marketing properties. Antimicrobial activity is one of the most intriguing features of cellulose fibers. Currently, cellulose fiber surface modification is regarded as one of the most effective methods for enhancing the durability of medical fabrics. For instance, cellulose reacts with methylol-5, 5-dimethylhydantoin coupled with hypochlorite to generate chloramine on the fiber's surface, which has antibacterial properties. The manufacture of ethyl cellulose nanocompounds employing spiro oxazine as a light stabilizer is another approach. During the high-temperature printing process, nanoparticles of cellulose preserve their photochromic characteristics without sacrificing their physical qualities.

CO₂ Capture

These nanomaterials, particularly active nanocellulose aerogels, can be employed to store CO₂ selectively as adsorbents or membranes. In this way, nanocellulose has been utilized to substitute physical hydrogen bonds or chemical bonds, such as borax, epichlorohydrin, or glutaraldehyde cross-linked hydrogel, which can be transformed to aerogel in ice-free cycles. CO₂ capture by chemisorption is enhanced by the modification of nanocellulose aerogels containing silane such as 3-amino propyl methyldiethoxysilane, 3-silane derivative, and N-2-aminoethyl-3-silane derivative. Utilizing fibrillated nanocellulose aerogels produced with acetate-functionalized crystalline nanocellulose suspension, CO₂ storage via physisorption was also done.

Challenges

Once implemented, specific applications for the extraction of nanocellulose from diverse biomass wastes support the efficacy of novel technology in the production of certain high-value goods. However, technological obstacles and financial concerns must be resolved prior to mass manufacture and utilization. The collection, transportation, and storage of biomass waste, as well as the use of expensive enzymes or chemicals, as well as high energy consumption, are the most expensive barriers. To achieve manufacturing on an economic scale, it is important to develop efficient methods to produce nanocellulose with outstanding characteristics, the same size at cheap, low energy consumption, low-cost chemicals, and with active enzymes. In terms of technological obstacles, the creation of effective pre-treatment and precise change of structure and function stand out.

Preparing high-quality nanocellulose, especially for biomedical purposes, necessitates a process that effectively removes contaminants while leaving the cellulose unfinished. Because of the inherent features of nanocellulose, such as bioinertness, hydrophilic property, and heat dissipation, precise performance is needed to improve the performance for a specific application. In addition, choices must be taken to assess the advantages and downsides of employing micro cellulose. For instance, the

reinforcement using cellulose nanofibers to paper production can increase their strength, but it may also limit the slurry drainage capacity, so decreasing the efficiency of production. Even though there are some disadvantages to using nanocellulose, its superior mechanical qualities, high altitude, environmental friendliness, natural decay, recycling, and non-toxicity attract an increasing number of researchers to develop end-product applications with added value.

Conclusions

Agro-industrial wastes can be solids, slurries, or liquids, all of which are potential sources of environmental pollution. Utilizing agro-industrial waste for a variety of community uses contributes to the valorization and enhancement of the waste's value-added. There are numerous methods for utilizing agro-industrial waste to create goods with additional value in the sectors of biomedical application, energy harvesting and storage, food and packaging, and electronic materials. From a waste biomass extraction standpoint, cellulose can be synthesized via bacterial fermentation. It is possible to extract cellulose from plants, algae, tunicates, and bacteria. The cellulose manufacturing pathway in bacteria involves a multistep reaction involving regulatory proteins, catalytic complexes, and specific enzymes via both static and dynamic fermentation processes. Waste extracts used as carbon sources in a fermentation medium. BC was created to obtain particular qualities to fulfill the product's property requirements. Engineering of BC is carried out by modifying BC through surface functionalization, addition of functional material, combination with nanoparticles, and composite with other polymers, resulting in the enhancement of BCN properties. Future research must focus on the exploitation of BC synthesis and extraction so that this technique can be broadly used for nanocellulose-based products.

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Biopolymers from Marine Prokaryotes

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P. Nisha, M. Thangavel, Anju Mohan, C. Jyothilekshmi, T. A. Raslana, and K. A. Razeena

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P. Nisha (✉) · T. A. Raslana · K. A. Razeena

Department of Biosciences, MES College, Marampally, Ernakulam, Kerala, India

M. Thangavel

School of Life Sciences, Nehru College of Arts & Sciences, Coimbatore, Tamilnadu, India

A. Mohan · C. Jyothilekshmi

S. S. V. College, Valayanchirangara, Ernakulam, Kerala, India

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Abstract

The marine ecosystem is a world of various microbial diversity having numerous bioresources that are relatively unexplored and require more studies for the isolation and identification of the natural novel products, especially biopolymers. Biopolymers are the polymers produced by living organisms, including bacteria, fungi, algae, and plants. The wide variety, renewable nature, lower toxicity, and unique structural and physicochemical behavior, etc., of biopolymers has gained attention in the scientific community and in various industrial sectors. These biopolymers from living organisms, composed of various monomers as building blocks for larger structures, bonded with covalent bonds. Most biopolymers are biodegradable materials. The biodegradable polymers are used in wide areas and have also resulted in the development of a variety of commercial products. Many of these biopolymers, such as polylactic acid, are naturally occurring polymers and poly beta hydroxybutyrate polymers are used as plastics; they may also be used as the need for polyethylene or polystyrene-supported plastics increases. The process of the synthesis of marine bacterial polymers and other prokaryotic biopolymer-based materials is influenced by marine environmental factors such as the microbial population, pH, and temperature. The natural biofilm producers are isolated from different marine habitats such as sediment, the Arctic, the Antarctic, deep sea, thermal vents, and cold ice, which have a higher quantity of bacterial exopolysaccharides.

Keywords

Exopolysaccharides · Biofilm · Bacterial EPS · Marine polymers · PLA · PHA

Introduction

Biopolymers are a type of polymers that are formed during/between the growth cycles of a living organism through various metabolic processes. Many of these biopolymers are discovered from prokaryotes and other natural sources. These biopolymers are biosynthesized or chemically synthesized from biomaterials by the organisms. Most of the biopolymers are derived from prokaryotes, mainly bacteria, plants, and fungi. Biological polymers attract more attention owing to their complexity, biodegradability, nontoxicity, abundance, biocompatibility, and their applications in various areas. Synthetic polymers such as plastics and other nonbiodegradable polymers cause environmental pollution. Around 140 million tonnes of synthetic nonbiodegradable polymers (man-made) are synthesized every

year for various purposes and cause major environmental issues. Biopolymers can be classified on the basis of various criteria such as origin, types, basis of degradability, number of monomeric units, heat responses, etc.

In prokaryotes such as bacteria, the intracellular polysaccharides are produced inside the cell as a cytoplasmic plasma membrane, whereas polysaccharides in bacterial cell walls act like a structural composition. Extracellular polymeric polysaccharides or exopolysaccharides (EPS) are covalently bound to the outer surface of a cell as capsule, sometimes these substances are loosely bound to cell surface as a form of slime. Most of bacterial cells capable for produce EPS in natural conditions to the extracellular surfaces and few microbes with greatly capable to secrete EPS in higher quantity when they are in stressful conditions (Papinutti 2010). Comparing with the production of hydroplant, bacterial EPS production is time saving i.e., take 2–3 days and the another advantage is that EPS production does not need land for cultivation and the waste products such as glycerols and hydrocarbon residues from industries are used as carbon substrates. These advantages in the production cost of EPS, is crucial factor that restraining the production and are industrially powerful substances. The main expenses are for the substrate required for the maximum growth of microbial cells on the bioreactor. Extensive studies are needed to optimize the ability of microbes to produce more EPS for the formulation of novel biocompounds, which is beneficial for multiple research areas. A comprehensive study is necessary to understand the physical and chemical properties of bacterial polysaccharides and could be more helpful for researchers in developing more useful compounds in various areas.

Biopolymers from marine sources such as bacteria and archaeobacteria, have novel biocompounds with higher biological activities and applications on a commercial level. The important characteristics of EPS from marine bacteria and the pigment produced by marine organisms such as carotenoids and melanins, can improve the survival/defensive mechanism of the organism. These biocompounds can be exploited for various products ranging such as adhesives, emulsifiers, and surfactants. Numerous marine biopolymers are significant, unidentified, unexplored, and undeveloped biological resources.

Bacterial Biofilm and Exopolysaccharides

In the marine ecosystems, microorganisms are the normal biota and are found in various areas in the oceans such as open oceans, marine sediments, marine organisms, and the other marine surfaces. The term ‘biofilm’ was first introduced by in 1978 by Corterson, and is a surface-attached (sessile) microbial community that are grown covered or surrounded by an EPS produced by itself. Biofilms are tightly packed communities of microbial colonies that can develop attached to a natural or synthetic surface, in an aquatic and soil environment, medical equipment, living tissues, water piping systems in potable and industrial usage, and are responsible for the formation of multicellular communities of biofilms (Dalton and March 1998). The colonization of bacterial cell on surfaces such as metal, concrete, biotic, and suspended particles, are survival strategies for microbes because this colonization gives more advantages in access to nutrients, protection from antibiotics and toxins,

maintaining the activities of extracellular enzymes, and protecting them from predation. The rates of the biofilm formation process vary widely depending on the type of microorganisms and environmental conditions such as the characteristic of a selected medium, temperature, pH, and ionic strength, factors that have an influence in changing the physicochemical properties of a bacterial surface and the microbial adhesion to the substrate (Hamadi et al. 2004).

As for the bacterial cells in a biofilm embedded in EPS, these extracellular polymeric substances comprise capsular polysaccharides, which produce a cohesive layer or capsule that is tightly linked to the cell surface, and the extracellular polymeric substances/ EPS secrete a layer of loosely attaching slime to the surface of the bacterial cells or into the outer environment. At the time of processing of the colonization on the surfaces, bacterial cells secrete extracellular polysaccharide substances that construct the biofilm matrix (Geesey and White 1990). Capsular EPS are produced mainly during the second phase of growth, i.e., the log phase, and slime EPS production mainly takes place during the stationary phase. Microbial cells commonly contain various polysaccharide structures and have a key role in maintaining shape and rigidity. Mainly they are able to produce polysaccharides characterized by the cellular location and their function. There are three mainly types:

- (a) Intracellular polysaccharides, are the storage form of carbon.
- (b) Cell wall polysaccharides such as teichoic acid and peptidoglycan.
- (c) Extracellular forms, EPS secreted in the growth medium and capsule in the outer side of the cell surface.

The outer capsular structure acts as a major protective cover of the cells from adverse environments such as toxic substances, osmotic pressure, temperature, oxygen tension, and may take part in the intake of the metal ions (Cerning 1990). The presence of EPS involved in biofilm formation also supports the interaction of bacteria. Almost all of the microbes are capable of producing a highly viscous layer surrounding the bacterial cell, which may give protection against phage attack or desiccation (Gruzdev et al. 2011) and some microbes are able to synthesize an EPS that gives the microbial cells protection from abiotic stress. EPS facilitate the free living organisms to attach and colonize on a biotic or abiotic surface where the nutrient components are accumulated. Some of the bacterial EPS are able to exhibit flocculating properties. Environmental factors such as heat shock, cold shock, acidic or alkali shock affect the productivity of secondary metabolites. Various studies have proved the involvement of environmental stimuli in the enhancement of the production of secondary metabolites. Microorganisms have the ability to adapt to the stressful situations in their natural environment.

A total of 32 bacterial species are isolated and identified based on physiological, biological, and molecular characteristics, mainly including the species of *Micrococcus*, *Klebsiella*, *Vibrio*, *Pseudomonas*, *Staphylococcus*, *Salmonella*, *Chromobacterium*, *E. coli*, *Halomonas*, *Bacillus*, etc. (Nisha et al. 2020). Some of the studies reported on the isolation and identification of bacteria from marine

biofilm attachment on the surfaces, isolated 11 bacterial strains from the ship hull, and identified morphological, biochemical, and molecular characteristics. Sravankumar et al. (2014) identified three genera of ten species, including *Bacillus* sp., *Vibrio* sp., and *Serratia* sp., from a marine biofilm attached to artificial surfaces (iron panels) based on Bergey. Marine organisms such as *Shewanella oneidensis*, *E. coli*, *Pseudomonas aeruginosa* (Lee and Newman 2003), *Vibrio proteolyticus* (Paul and Jeffrey 1985) and *Bacillus subtilis* (Omoike and Chorover 2004) were able to be involved in the process of biofouling. Marine bacterial species such as *Micrococcus*, *Halomonas*, and *Bacillus*, isolated from marine sediment, were studied by Abdelnasser Salah Shebl Ibrahim (2008). Most of the organisms that were subjected to screening were capable of producing biofilm on the steel surfaces except for some of them. *Micrococcus luteus* produced more biofilm than any other of the study organisms. A similar study reported that a marine *Micrococcus* sp. was able to attach onto steel surfaces (Nisha and Thangavel 2014). Attachment is the primary process in biofilm formation and the density of biofilm is dependent on the surface. Biofilm producing 82 bacterial species from the ship hull (MV-Balavan) at Cochin, Kerala. Another biofilm producer was analyzed using the crystal violet binding assay method and was identified as *Micrococcus luteus* by 16SrRNA sequencing (Nisha et al. 2020).

The bacterial EPS acts as a mechanical barrier between some bacteria and defense compounds of plants such as polymers from *Pseudomonas syringae* pv. *phaseolicola* and *Sinorhizobium meliloti*, which can be resistant against reactive oxygen species secreted by host plants at the time of infection (Lehman and Long 2013) and decrease oxidative stress. In some bacteria, polymers are involved in virulence and in pathogenicity. *Erwinia amylovora* shows high virulence owing to the production of levan and amylovoran and also decrease plant colonization. A study on *Rhizobium* NZP2037 reported that it is able to use its own poly-beta hydroxybutyrate and use EPS in carbon-restricted situations as a whole source of carbon and survived (Patel and Gerson 1974). The chemical characteristics of EPS such as pH, surface properties, metal content, ionic content, play a key role in metal biosorption (Fukushi 2012). These EPS can accumulate the other nutrients when grown under conditions of an additional supply of carbon. *Paenibacillus jamilae* produce EPS, and its biosorption capacity on various metals was studied and found to adsorb heavy metals such as Cd, Cu, Zn, Ni, Co, and Pb, and have maximum interaction with PB and a higher binding affinity of 303.03 mg/g (Morillo Pérez et al. 2008). Some of the studies on remediation in heavy metal areas for the recovery of the polluted environment revealed the capability of bacterial EPS on metal absorption. These microbial EPS are also capable of binding, absorbing or trapping some metal ion colloids, clay minerals, oxides, etc. The EPS from *Azotobacter chroococcum* XUI is capable of absorbing 47.87 and 40.48% Hg and PB respectively (Rasulov et al. 2013). The bacterial polymer xanthan showed Cu absorption with unusual biosorption and decreased pH 3.5 to 5.5 (Causse et al. 2016). Most of the bacterial EPS are composed of various components such as carboxyl groups and hydroxyl functional groups, EPS are negatively charged owing to these components (Ding et al. 2018). Another study on polymers from *Nostoc muscorum* and *Anabaena*

variabilis showed biosorption with Cu, Co, Zn, Cd, and Ni, with maximum affinity for Cu. Similarly, the EPS produced *Pseudomonas* sp. CU-1 to protect the cells from heavy metals because it has an ability to bind with Cu (Lau et al. 2005).

Microbes producing EPS got an advantage from the stress of drought environmental conditions. The EPS from *Pseudomonas* spp. from soil have a higher water-holding capacity and had altered moisture when added to sanded soil (Roberson and Firestone 1992). Some cyanobacteria, including *Nostoc calcicola* and *Phormidium* 94a, were isolated from a desiccated area producing EPS, which is also capable of nutrients, water retention, and a survival mechanism (Bhatnagar et al. 2014). EPS produced from *Planococcus rifietoensis* RT₄ and *Halomonas variabilis* HT₁ were inoculated into chickpea plants and various factors were studied. The results showed increased plant growth, that soil aggregation improves more than 75% in elevated salt stress, and protected the plants from salinity (Qurashi and Sabri 2012). Bacterial EPS produced by plant-associated organisms was also able to involve the plants in salt stress tolerance. The biopolymer from NaCl-tolerant bacterial species can decrease the uptake quantity of Na by the plants by decreasing the amount of ions (Upadhyay et al. 2011) and preventing osmotic stress, which can also promote the survival of microbes and is useful for plants. Bacterial EPS protect the cells from desiccation/drought like a protective soft sponge and show structural modifications during desiccation and can trapped nutrients and water, also act as a protecting factor for the survival of the microbial cells (Roberson et al. 1993).

Microbial extracellular polysaccharides can protect the thermophiles, they cannot dissolve at high temperatures, and acts as shielding for the microbes. These characteristics were studied in thermophiles such as *Geobacillus tepidamans* V264 and *Bacillus* sp. B3-72 (Kambourova et al. 2009). Microbes producing EPS can alter the microstructure and desalination and improve the habitability and survivability of the microbes (Krembs et al. 2011). These extracellular polymeric substances from archaea and thermophiles have a unique slime layer/EPS with a large number of potential applications in various industries. Microbes living in sea-ice can produce EPS, play a key role in cryoprotection, and have adaptations at low temperatures and also high salt concentrations. Higher EPS production was shown by organisms isolated from Arctic sea-ice and they are capable of protecting the microbial cells from harsh environmental conditions (Caruso et al. 2018). Extracellular polymeric substances can be a protection factor for thermophilic bacteria by shielding microorganisms from very high temperatures. The polymers produced by *Bacillus* sp. strain B3-72 and *Geobacillus tepidamans* V264 are not easily dissolved at high temperatures (Nicolaus et al. 2000). Biofilm producers have lower susceptibility to antimicrobials than the microbes than free living ones. These biofilms possess negative charge and are capable of binding with positive charges molecules and protect the cells. Numerous studies reported the potential capacity of bacterial EPS against antimicrobial agents and pathogens. The EPS produced by *Acinetobacter baumannii* have antibiotic sensitivity against tobramycin (Davenport et al. 2014) and a similar study on slime/EPS from *Staphylococcus* spp. showed effective antagonistic activity against pefloxacin, tobramycin, and teicoplanin (Souli and Giamarellou 1998) (Table 1).

Table 1 Identification of biofilm producers. (Based on Bergey's Manual)

S. No	Name of Sample	Grams Reaction	Motility	Spore	Colony Morphology		MC	Pigmentation	Organism
					NA	NLF			
1	MRN1	+, Cocci	—	—	Medium, translucent	NLF	NLF	Pale orange	<i>Micrococcus</i> sp.
2	MRN2	+, Cocci	—	—	Small, pinpoint, translucent	LF	LF	Golden yellow	<i>Staphylococci</i> sp.
3	MRN3	+, Cocci	—	—	Small	LF	LF	—	<i>Streptococci</i> sp.
4	MRN4	—, Rod	—	—	Glistening	LF	LF	White	<i>Enterobacter</i> sp.
5	MRN5	—, Rod	—	—	Medium	NLF	NLF	Red	<i>Serratia</i> sp.
6	MRN6	—, Rod	—	—	Thin	NLF	NLF	Pale gray	<i>Shigella</i> sp.
7	MRN7	—, Rod	+	—	Diffused growth	NLF	NLF	Green	<i>Pseudomonas</i> sp.
8	MRN8	—, Rod	+	—	Swarming	NLF	NLF	—	<i>Proteus</i> sp.
9	MRN9	—, Rod	—	—	Small	LF	LF	—	<i>Aeromonas</i> sp.
10	MRN10	—, Rod	+	—	Medium	NLF	NLF	—	<i>Alcaligenes</i> sp.
11	MRN11	+, Cocci	—	—	Small translucent	NLF	NLF	—	<i>Halococcus</i> sp.
12	MRN12	+, Cocci	—	—	Medium, translucent	NLF	NLF	Yellow	<i>Micrococcus</i> sp.
13	MRN13	—	—	—	Medium, mucoid	NLF	NLF	—	<i>Acinetobacter</i> sp.
14	MRN14	+, Rod	+	+	Large, opaque, white	NLF	NLF	—	<i>Bacillus</i> sp.
15	MRN15	—, Rod	—	—	Large	NLF	NLF	Yellow	<i>Flavobacterium</i> sp.
16	MRN16	+	+	+	Large, rhizoid	NLF	NLF	—	<i>Bacillus</i> sp.
17	MRN17	+, Cocci	—	—	Medium	NLF	NLF	Bright Yellow	<i>Micrococcus</i> sp.
18	MRN18	—, Rod	+	—	Small, translucent	LF	LF	—	<i>Salmonella</i> sp.
19	MRN19	—, Rod	+	—	Small	LF	LF	—	<i>Enterobacter</i> sp.

(continued)

Table 1 (continued)

S. No	Name of Sample	Grams Reaction	Motility	Spore	Colony Morphology		Pigmentation	Organism
					NA	MC		
20	MRN20	–, Rod	–	–	Medium	LF	–	<i>Shigella</i> sp.
21	MRN21	–, Coccobacilli	–	–	Medium, shiny	NLF	Dark violet	<i>Chromobacterium</i> sp.
22	MRN22	–, Rod	+	–	Small, translucent	LF	–	<i>E.coli</i>
23	MRN23	–, Rod	–	–	Large, mucoid	LF	–	<i>Klebsiella</i> sp.
24	MRN24	–, Rod	–	–	Medium	NLF	Orange	<i>Shewanella</i> sp.
25	MRN25	–, Rod	+	–	Small, translucent	NLF	–	<i>Halomonas</i> sp.
26	MRN26	+, Rod	+	–	Medium, mucoid	LF	–	<i>Klebsiella</i> sp.
27	MRN27	–, Rod	+	–	Irregular	NLF	Gray	<i>Pseudomonas</i> sp.
28	MRN28	–, Coccobacilli	+	–	Small, shiny	NLF	Violet	<i>Chromobacterium</i> sp.
29	MRN29	+, Rod	+	+	Medium, opaque	NLF	White	<i>Bacillus</i> sp.
30	MRN30	–, Rod	+	–	Small	LF	–	<i>E. coli</i>
31	MRN31	+, Cocci	–	–	Pinpoint	LF	Golden yellow	<i>Staphylococci</i> sp.
32	MRN32	Comma	+	–	Small	LF	–	<i>Vibrio</i> sp.

NA, nutrient agar; MC, MacConkey agar; LF, lactose fermenting; NLF, nonlactose fermenting

Physical and Chemical Properties of Biopolymers

Polymers or macromolecules from microbes are classified by different possibilities such as by source, structure, synthesis, and molecular forces. These macromolecules are high molecular compounds 10,000 to 10,000,000 Da in mass. The polymers are mainly of two major groups of polysaccharides depending upon structural unit, homopolysaccharides, which consist of only one sugar component, and heteropolysaccharides, which consists of two or more sugar components.

Also, there are two types based on source, natural polymers, e.g., nucleic acids, proteins, polysaccharides, and natural rubbers, and synthetic polymers, e.g., Teflon, polyethylene, PVC, and polystyrene.

The biopolymers are classified based on structures in to three groups: linear polymers, where monomers are joined to form a straight long chain, owing to the close packing of polymer chains (these polymers have high tensile strength, a high melting point, and high densities; branched chain polymers, where monomer units are combined to produce a main chain or a linear chain that also forms a branch along the main chain; cross-linked polymers, three-dimensional (3D) networks polymers, where a linear chain is initially formed, which are joined together to form cross-linked polymers.

Depending on the molecule formed the polymers are of two types, globular and fiber, and by the nature of the atom, polymers are classified in to carbon-containing polymers, heteropolymers, organic element, and inorganic polymers.

The biopolymers from bacteria are in various classes such as polysaccharides, composed of sugars with glycosidic linkage; polyamides, composed of amino acids with peptide bonds; polyesters, composed of a hydroxy fatty acid linked by ester bonds; polyphosphates, composed of inorganic phosphates linked with anhydride bonds.

Bacterial polysaccharide synthesis consumes nutrients and chemical energy, is maintained by bacterial cells, and are responsible for growing and persisting in unfavorable conditions. Bacterial polymers that are high-molecular-weight polymers are responsible for forming helical strands, are present commonly in a double-stranded forms such as xanthan, kappa carrageenan, gellan, and succinoglycan, or otherwise triple-stranded structures such as curdlan and *Schizophyllum* (Nishinari and Takahashi 2003). Various condensation linkages in polysaccharides with variations of monomeric sequences give a wide range of structural architecture and shapes. Sometimes, polymer chains are in high-molecular-weight substances, which results in complex determined physical properties. The common monosaccharides in the bacterial cells are D-glucose, D-galactose, D-mannose, L-fucose, L-rhamnose, and N-acetyl hexosamines, and N-acetyl D-galactosamine. In some organisms, uronic acids such as D-glucuronic acid could be present. Many other sugars are present in microbial polysaccharides, such as acetate with ester linkage or pyruvate linked with ketal, and influence the structural and physical characteristics of polymeric substances. Other ester-linked substituents such as propionyl, succinyl ester, hydroxybutanyl, glyceryl residues, and a few of the amino acid substituents

have been characterized in microbes (Sutherland 1998). Ester-linked O-acetyl groups are the organic substituent in bacterial polysaccharide in some microbes. Ketal pyruvate is also one of the important constituents in bacterial polysaccharides that are imparted in the charge of these polymers. Knowledge of the physical and chemical properties and composition of polysaccharides from microbes could help to exploit and invent new hydrocolloids in various areas such as cosmetics, food, and biomedical applications. Bacterial polysaccharides that have been industrially commercialized include gellan gum (*Sphingomonas elodea*) (Gonçalves et al. 2009), dextran (*Leuconostoc mesenteroides*) (Mollet 1996), and succinoglycan (*Rhizobium* sp.) (Chouly et al. 1995).

Types of Biopolymers in Prokaryotes (Bacteria)

The historical evidence on polymers is from 1886, the finding of a cellulose biomaterial produced by a bacterial species was reported, the first polymer from bacteria *Leuconostoc mesenteroides*, is dextran, which was found in a microbial product. Louis Pasteur discovered this in the mid-nineteenth century (Van Tieghem 1878). After discovering EPS, the first intracellular polymer-like polyamide cyanophycin was discovered (Borzi 1887). After 40 years, polyhydroxybutyrate was found in *Bacillus megaterium* (Lemoigne 1926) and some of the other polymers with medical industrial applications were discovered from various bacterial species in the twentieth century such as alginates (Linker and Jones 1966), xanthan (Leach et al. 1957), polyglutamate (Ivanovics and Erdos 1937) and polyphosphate (Kornberg et al. 1956). These, started a new trend in research into diverse biomaterials. After these discoveries of biopolymers, overall research interests into the purification, different biological activities and synthesis, and metabolic activities were also studied. These areas of research were at a molecular level such as DNA sequencing, gene clusters, cloning of genes, identification analysis of genes of biopolymers between 1970 and 2000. As continuation, the reactions involved in mechanisms of particular enzymes including, synthases, synthetases, polymerases, and the functions of proteins, co-polymerases were studied. Various types such as intracellular and extracellular biopolymers were isolated and characterized by researchers from the through the decades, whereas numerous organisms with high potential novel biopolymers should be exploited. Biopolymers such as starch, glycogen, polyphosphate, polyhydroxyalkanoates (PHAs), etc., are accumulated in cell cytoplasm. There is a limitation to the availability of space in cytoplasm, which limits the amount of biopolymer produced by an organism. Many of the polysaccharides such as dextran, curdlan, xanthan, alginates, pullulan, chitosan, cellulose, and poly (β -D glutamate) are the polymers secreted extracellularly. Polylactic acid and PHAs, has been produced on a large scale for biotechnological applications.

Xanthan

Xanthan is a copolymer with a complex structure produced by some bacterial species as a part of metabolism and is the first commercially produced bacterial polysaccharide through fermentation. These xanthan polymer building blocks mainly contain five different sugar groups produced by *Xanthomonas campestris*. Xanthan is used extensively in food items such as cheese spreads, ice creams, deserts, and puddings as a gelling agent and in nonfood industrial applications such as the mineral ore processing, oil recovery, agriculture, paper manufacturing, cosmetics, and pharmaceutical sectors.

Dextran

Dextran is a polysaccharide with a large family of bacterial polysaccharides that polymerized outside of the cell by an enzyme called dextran sucrose. It has monomers of simple sugars and is stored in bacteria and in yeasts. These polymers have various medical applications, including blood volume expanders, wound covering, improvement of blood flow, wound coverings, surgical sutures, and in the treatment of anemia. Dextran hemoglobin compounds may be used as blood substitutes owing to the capability as plasma expanders and ability to deliver oxygen.

Cellulose

Cellulose is a complex structural sugar compound and can be produced by some bacterial species such as *Cellulomonas* and *Micrococcus*, etc., having unique properties. Cellulose is the homopolymer unbranched β (1,4)-linked glucose units. *Salmonella enterica*, *Rhizobium* spp., *Agrobacterium* spp., and *Pseudomonas aeruginosa* produce cellulose polymer. Bacterial cellulose has Generally Recognized As Safe (GRAS) status and is widely used in biomedical and packaging products and also in food. Bacterial cellulose has a very large surface area and is able to absorb liquids. A very low quantity of bacterial cellulose can be used as thickening, binding, and coating agents.

Pullulan

Pullulan is a polymer made by repeating units of maltotriose residues with α -1,6 glycosidic bonds. Microbial pullulan secretes extracellularly to the outer surface as EPS. Pullulan is a water-soluble polysaccharide produced by some species of *Aureobasidium pullulans*. Pullulan is a biodegradable, nontoxic, odorless, tasteless, and bioactive polymer. The main application of pullulan is the use as a food additive

that is in low-calorie foods and drinks, and it can also be used instead of starch. The pullulan biopolymer can be used as a flocculating agent for the precipitation of uranium clays, potash clays, and in mineral ores. Pullulan compounds can serve as drug carriers in the medical field, pullulan can work as a plasma expander without undesired side effects and is completely excreted out after metabolic conversions. Pullulan compounds can also serve as medical adhesives.

Glucans

Glucans are mainly produced by *Saccharomyces cerevisiae*, and are a homopolymer of the simple sugar glucose. Glucans are purified from yeasts and are used as immunomodulators, antiviral agents in plants, and inhibit tumor growth. *Schizophyllum*, lentinan, and grifolan are isolated from *Schizophyllum commune*, *Lentinus edodes*, and *Grifola frondosa* respectively, are β (1–3) (1,6)-glucans, and are effective as antitumor agents.

Glycogen

Various types of bacteria produce polysaccharides and excessive polysaccharides are stored as glycogen in the cells. These polysaccharides show water solubility with a 1,4-glycosidic bond in linear and α 1,6-glycosidic bonds in side chains. In bacterial cells, glycogen is accumulated as in limited growth conditions with excessive carbon sources and other nutrients are in deficient quantities. This glycogen is a common potential indicator of a bacterial lifestyle.

Alginate

Alginates are nonrepeating, negatively charged heteropolymers with β -(1,4)-linked β -D-mannuronic acid and guluronic acid. Alginate acts as a survival mechanism by protecting the bacterial cells from phagocytes when the cells are in a biofilm matrix, e.g., *Pseudomonas aeruginosa*. These polymers interact with divalent cation and form dense hydrogels with a high water-holding capability. The EPS produced by *Pseudomonas aeruginosa* also enhance bacterial survival in chlorinated water. Bacterial alginates are widely used as biobased materials in food and for pharmaceutical and cosmetic purposes, for example. Alginates produced by *Pseudomonas aeruginosa* are able to protect bacteria against inflammation in hosts, phagocytosis, etc.

Hyaluronic Acid (Hyaluronate)

Hyaluronates are negatively charged, unbranched heteropolymers with β -(1,4)-linked repeating heteropolymers of glucuronate and N-acetyl glucosamine. It is a

component of biofilm and has antiphagocytic action. Hyaluronates are found in *Streptococcus* spp., *Pasteurella*, and *Bacillus cereus*.

Polyesters

Polyesters are PHAs, highly reduced biopolyesters, and function as an electron sink in an anaerobic zone of biofilm. Some of the PHAs, such as polyhydroxybutyrates are naturally found polyesters formed into spherical inclusions with hydrophobicity.

Polyamides

Polyamides are highly charged polycationic (ϵ -PL) and polyanionic (γ -PGA) materials and are nontoxic, biodegradable, and renewable. Polyamides are composed of amino acids and are produced by synthetases. Some of the nonpathogenic organisms such as *Bacillus megaterium* and *B. licheniformis* produce polyamide-based materials. Polyamides function as capsules or slime layers in bacterial cells to protect them, and also act as intracellular storage materials. In industries, polyphosphates are used as substitutes for chemical polymers.

Polyphosphate

Polyphosphates are chains with condensed phosphates that functions as a storehouse with negatively charged, high-energy rich bonds. The metabolic activity of this polyphosphate is positively related to the virulence factor production of the microbe. These polyphosphates function as phosphate storage molecules, acts as buffers, and give energy to the metabolic pathways. Polyphosphates regulate cell signaling and are involved in growth, viability, virulence, and stress tolerance, etc.

Curdlan

Curdlan is a bacterial EPS, comprises β -(1,3)-glucan, has various applications. These materials are insoluble in water, and have gelling activity. This biopolymer is approved by the FDA for safe use as dietary fiber in Taiwan, Korea, and Japan, and also as food additives. The fermentative production of curdlan on a commercial scale uses *Alcaligenes faecalis* var. *myxogenes*, reclassified as *Agrobacterium* spp.

Gellan

Gellan is an extracellular polysaccharide with high molecular weight, composed of 1,3-beta found in *Sphingomonas elodea*, *S. paucimobilis*, and *Pseudomonas elodea*. Two main types of gellan are used. “KELCOGEL” is an industrial food product and purified “Gel-Gro” gellan gum is used in the pharmaceutical and biomedical fields.

Prokaryotic (Bacterial) Biopolymers from Various Marine Sources

Marine bacterial EPS contain heteropolysaccharides with various monosaccharides such as pentoses (D-ribose, D-arabinose, D-xylose), hexoses (D-glucose, D-mannose, D-galactose, D-allose, L-rhamnose, and L-fucose), amino sugars (D-galactosamine, D-glucosamine) or uronic acids (D-glucuronic acid, D-galacturonic acids). Other inorganic substances such as phosphates, sulphates, succinic acids, acetic acids, and pyruvic acids may also be present. The marine polymers with other macromolecules such as lipids, proteins, and nucleic acids, comprise the organic matrix present in the intracellular space of bacterial biofilms, and act as the largest reservoir of reduced carbon. In recent years, the marine habitat has attracted great attention, especially in extreme environments and extremophiles has raised its peak. Several studies have reported on ice cold microbial marine communities, and found that these organisms produce EPS and play a key role in cryoprotection. The production quantity of EPS as a capsular form in bacterial pathogens. The production rate and the amount of EPS accumulated influences the pathogenicity of an organism.

Exopolysaccharides play a key role in biofilm matrix production by bacterial cells, and are involved in cell-to-cell communication in between microbial cells and its close cells. The nutrients also interact with EPS to increase the rate of intake of substances/molecules, dissolved compounds, and support microbial growth. Hydrated biofilm acts as stable microenvironment and here, the extracellular enzymes can find storage and ease the uptake of molecules. Bacterial strains from deep sea hydrothermal vents showing resistant to heavy-metal substances and the purified form of EPS have the capability to bind the metals and toxic substances (Wuertz et al. 2000). These EPS exhibit the polymeric state in the marine environment and show binding affinity toward cations and trace metals. EPS isolated from Antarctic regions and sulfate groups act as anchoring agents for cations, and are found as trace metals in the Southern Ocean (SO). This improves the bacterial communities in areas with limited availability of trace metals such as iron (Mancuso Nichols et al. 2005).

Exopolysaccharide from *Alteromonas* strain 1644 are found in annelids in deep sea thermal vents, and showed a low viscosity at a low ionic concentration (Samain et al. 1997). This organism has the ability to secrete two types of polysaccharides and are differentiated by their gelling property. Marine bacteria are capable of higher production of various EPS when growing with limited quantities of nutrients such as phosphorus and nitrogen under laboratory conditions. *Hahella chejuensis*, from the marine sediment of Marado, Republic of Korea, can produce the highest yield of EPS in sucrose-enriched culture media (Ko et al. 2000), whereas haloalkaliphilic bacteria from saline lakes in Antarctica, *Halomonas alkaliantartica* CRSS, was a potentially greater producer of EPS in the presence of acetate as a carbon source (Poli et al. 2004). Microbes from volcanic hydrothermal vents, deep sea hydrothermal vents, and shallow thermal springs have a novel EPS producer, *Pseudoalteromonas* strain 721, producing EPS with octasaccharide repeating units with

two side chains. These EPS show gel formation and viscoelastic behavior at increasing temperature (Rougeaux et al. 1999).

In a study on CMG607w and CMG1421 two strains of *Pseudomonas aeruginosa* produce commercially usable biopolymers. CMG607w was from the sediment of the Lyari River outfall of the Arabian Sea, and can produce the medium-chain-length PHAs. PHA production was increased with an increase in incubation period (Jamil and Ahmed 2008). PHA has a fascinating source of various biomolecules and are highly potential interest to several industrial applications. Marine sponge and bacterial interactions are very complex, Nowadays, these mutualistic relationships attract attention because of the production of a bacterial biopolymer (polyesters) such as intracellular PHAs by the bacterial cell. These PHAs act as a carbon, an energy reservoir, and as the alternative to the fossil fuel-based polymers. The PHA production by these sponge-associated microbes are under stressful sponge mesophyl microenvironments. In this review, the researchers try to explore the industrial outlook for marine bacteria for the synthesis of PHA at a commercial level (Ganesan Sathiyarayanan et al. 2016). PHA production is highly expensive compared with petrochemical-based plastics and is too hazardous to the ecosystem. Easily and cheaply available carbon sources/raw materials such as coconut cake and rice bran are used to overcome this issue. The potential PHA-producing bacterial strains M6 and S6 are isolated from different seaweeds, and identified by 16SrRNA sequencing as *Bacillus cereus* RBL6 and *Pseudomonas pseudoalcaligenes* RBL7 respectively (Ramya et al. 2017).

Many of the bacterial species produce EPS as a survival mechanism in adverse conditions, a strategy for growth, and to adhere to solid surfaces. New research is ongoing in the area of the isolation of EPS-producing bacteria from the marine environment, especially from extreme environments such as the deep sea hydrothermal vents with high temperature, pressure, and the presence of heavy metals. Various marine EPS-producing bacterial species are also isolated from several extreme niches such as cold environments, especially Arctic and Antarctic sea ice with extreme environmental conditions such as temperature, nutrient concentration, and salinity in marine, aquatic, and terrestrial ecosystems. Bacterial EPS from these extreme environments are heteropolysaccharides that have three to four different monosaccharides. This work gives an overview of current knowledge on EPS-producing marine organisms has a symbiotic association with marine annelid worms that were living in an extreme niche (Poli et al. 2010). The psychrophilic bacterial isolates from South Ocean produce their EPS as an extracellular response to the environmental constraints. These EPS production responses of bacterial species in relation to cold and sea environments were explained.

In the study on marine *Micrococcus luteus* EPS and Fourier transform infrared (FTIR) results observed the presence of carboxyl groups, glucans, O-H, NH₂, C=O, C=N groups, etc. (Nisha et al. 2020). Various studies were reported on the spectral analysis of EPS from microbial strains. In EPS from *Micrococcus luteus* analyzed using FTIR, the results showed various peaks, a broad stretched peak 3423 cm⁻¹ has the presence of a hydroxyl group. The weak band at 2925 cm⁻¹ revealed the C-H

group, and a peak at 1739 cm^{-1} indicated the presence of uronic acid. Overall results reveal the presence of the carboxyl group and uronic acid (Patil et al. 2009). A similar study reporting an *α -Proteobacterium* group included the functional group from crude EPS, a broad band at 4000 cm^{-1} to 650 cm^{-1} with a resolution of 4 cm^{-1} and a sharp band at 2989.89 cm^{-1} , indicating the presence of O-H, carboxylic acid, and H-bonded groups. C-O, alcohol, ether, and phenol groups are observed from the peak at 1038.92 cm^{-1} (Sunil Pawar et al. 2013). Ortega Morales et al. (2007) studied EPS and reported that the existence of ^-OH (2800 cm^{-1} to 3600 cm^{-1}) groups, also COOH groups (1600 cm^{-1} to 1725 cm^{-1}). B3 strain displayed the occurrence of an OH band at 3415 cm^{-1} and the presence of a band at 1631.48 cm^{-1} (Ashok Kumar et al. 2011). Similar results are reported by Anita Iyer et al. (2005): the FTIR result of EPS produced from marine *Enterobacter cloacae* revealed the existence of O-H, COOH, C-H, C-O, S=O groups. Bragadeeswaran et al. (2011) studied EPS from *Bacillus cereus* GU 812900, with peaks at 2853.98 to 2923.88 cm^{-1} , the presence of C-H stretching in an aliphatic compound in the cell wall such as carbohydrates and fatty acids is identified and spectral peaks at 1645.79 cm^{-1} showing the presence of an amide II band. Another peak at 1560.53 cm^{-1} revealed the NH_2 bending, C=O, C=N stretching amide I band. The results showed (1, 3) glucan linkages, the values are close to 1000, 2000, 3000, 15,000, and 1600 cm^{-1} (Vijayabaskar et al. 2011). Related work studying the FTIR results of *Paenibacillus polymyxa* JB115-BG showed the peaks at 3420 cm^{-1} O-H stretching, 2938 cm^{-1} C-H band, and 1634 cm^{-1} indicates C=O bond (Jung et al. 2007), and a similar result was reported, which revealed the peaks to have protein conjugated glucan at 1242 cm^{-1} (Gonzaga et al. 2005).

The microbes live in deep marine environment and can survive under extreme ecological and harsh environments such as high salinity, low temperature, and high atmospheric pressure. These characteristics lead them to be of scientific interest. Banerjee et al. (2019) studied the microbes from the SO, a deep marine ecosystem with a diverse group of microbes. They isolate EPS producers, optimize the production of EPS and characterize EPS, results shown that, three different EPS showing structural confirmation, having porous structure to powerful flakes same as polymeric compounds with C:N ratio between 4 to 11. The FTIR characterization showed the existence of carbohydrate, water molecules, and protein-associated amides. EPS of a microbe are biopolymeric in nature and help in biofilm formation, providing a protection layer to microbes by forming a complex interrelationship between cells and entrapping the nutrients (Di Donato et al. 2016). In this way they assist bacterial microcolonies to withstand extreme environmental conditions and allow easy accessibility of nutrients between bacterial cells. Marine prokaryotic organisms produce biopolymers with an important property as biomaterials with suitable industrial materials. By examining these structural and physicochemical properties it was found that they had a large field of possibilities such as pharmaceuticals, food processing, bioremediation of petrochemical spillage, etc. Gray and Biddlestone (1973) reported that *Halomonas hydrothermalis* MB-45 is a good EPS producer, isolated from the SO (Indian Sector) and the higher production of 5.26 g/L and with a carbon nitrogen (C/N) ratio between 4 and 11. A similar study on

Pseudoalteromonas spp. from the SO showed the same range of C/N ratio. The surface morphology of the EPS-producing *Zunongwangia profunda* MB24 had similarities to *Pseudoalteromonas* spp., having porous and smooth regions in the EPS (Sengupta et al. 2019). This EPS was polysaccharide macromolecules with α -glycosidic bonds, polysaccharides, and amine-substituted. These compact structures indicate their potential applications as thickener, viscosifier, stabilizing agent, etc. The porous characteristics of EPS have been proven to be a key factor for heavy metal biosorption and has the ability to hold more water. The fibrous pores of bacterial EPS help the cells to attach to each other with high-affinity interactions. The FTIR spectra of three bacterial species showed a functional group in polysaccharide macromolecules. Here, EPS from *Marinobacter algicola* MB-44 showed highly complex aminated branching polysaccharides (Fan et al. 2012). The bacterial EPS of SO origin have different applications in food industries as anticoagulant, thickening agent, biodegradation for waste water treatment, and anti-HIV activity, as well as having UV protection capacity.

Alteromonas macleodii subsp. *Fijiensis* is a potent EPS producer, which was isolated from the hydrothermal vent at a depth of 2600 m in the North Fiji Basin (Rougeaux et al. 1998). A valuable source of bacterial EPS was isolated from volcanic and hydrothermal marine areas including *Methanosarcina*, *Haloferax*, *Haloarcula*, *Sulfolobus*, *Bacillus* spp., *Thermotoga maritima*, and *Thermococcus litoralis* (Maugeri et al. 2002; Manca et al. 1996) and their biopolymers were studied. The thermophilic bacteria *Geobacillus* spp. from Phlegraean in Italy are found in shallow hydrothermal vents of the marine area and determined as a higher EPS producer (Nicolaus et al. 2002). Similarly, EPS production and characterization was done by using a thermophilic strain of *Geobacillus* sp. isolated from the marine sediment from a hot spring near the seashore of Maronti, Italy. Previous studies demonstrate the bacterial populations in Port di Livante, where the habitat was deep, and the hydrothermal vents of these communities. The studies reported that those bacteria are thermophilic, chemosynthetic, archaeal strains (Gugliandolo and Maugeri 1998). Thermophilic aerobic microbe can compete to make two exopolysaccharides (EPS-1 and EPS-2), isolated from a marine hydrothermal vent at Vulcano Island, Italy. The thermotolerant *Bacillus licheniformis* strain (B3-15) was isolated from a shallow marine hydrothermal vent at Vulcano Island, and is capable of producing EPS with immunomodulating properties, with a maximum production of 165 mg/L EPS on a liquid mineral medium (Maugeri et al. 2002) (Table 2 and Fig. 1).

Functions of Bacterial Biopolymers/Exopolysaccharides

Bacterial EPS is getting in this decade because of the rheological properties and the fermented food products (Cerning and Marshall 1999). Bacterial EPS have quite a lot of medicinal applications, such as antiviral and antitumor activity, lowering the level of blood cholesterol, and they also act as immunostimulation agents such as polysaccharide vaccine. With unique structural and physical properties, microbial

Table 2 EPS producing organisms from marine sources

Microorganism	Source of environment	Application	References
<i>Haloferax mediterranei</i>	Mediterranean Sea	Oil recovery in high salinity areas	Anton et al. 1988; Parolis et al. 1996
<i>Halomonas alkaliantartica</i> strain CRSS	Salt lake in Cape Russell in Antarctic	High viscosity	Poli et al. 2004, 2007
<i>Hahella chejuensis</i>	Marine sediment, Marado, Cheju Island, Republic of Korea	Biosurfactant, detoxifying agent in petrochemical oils	Lee et al. 2001
<i>Pseudoalteromonas</i> strain SM9913	Deep sea sediment in Bohai Gulf, Gulf of Yellow Sea, China	Flocculating and biosorptive agent	Qin et al. 2007; Li et al. 2008
<i>Pseudoalteromonas</i> strain CAM025	Melted Antarctic Sea	Cryoprotection	Mancuso Nichols et al. 2004
<i>Colwellia psychrerythraea</i> strain 34H	Arctic marine sediments	Cryoprotection	Mancuso Nichols et al. 2004
<i>Pseudoalteromonas</i> strain CAM 036	Southern Ocean	Trace metal binding	Mancuso Nichols et al. 2004
<i>Alteromonas macleodii</i> subsp. <i>fijiensis</i>	Deep Sea hydrothermal vent, North Fijian Basin	Waste water treatment, biotoxification, thickening agent, bone healing	Rougeaux et al. 1998; Zanchetta and Guezennec 2001
<i>Pseudoalteromonas</i> strain 721	Deep sea hydrothermal vent	Gelling agent	Rougeaux et al. 1998; Zanchetta and Guezennec 2001
<i>Thermococcus litoralis</i>	Shallow submarine thermal spring	Biofilm formation	Rinker and Kelly 1996
<i>Geobacillus</i> strain 4004	Marine sediment in hot spring from seashore, Maronti, Ischia Island, Italy	Pharmaceutical application	Nicolaus et al. 2002
<i>Micrococcus luteus</i>	Ship hull Arabian Sea, Port Cochin, Kerala, India	Biofilm formation, antimicrobial agent	Nisha et al. 2020
<i>Bacillus thermodenitrificans</i> strains B3-72	Hydrothermal vent, Vulcano Island, Italy	Immunomodulator and antiviral agent	Gugliandolo and Maugeri 1998; Arena et al. 2009
<i>Zunongwangia profunda</i> MB-24	Southern Ocean (Indian Sector)	Biofilm formation	Banerjee et al. 2019
<i>Marinobacter algicola</i> MB-44	Southern Ocean (Indian Sector)	Biofilm formation, viscosifier, thickener, and stabilizing agent	Banerjee et al. 2019

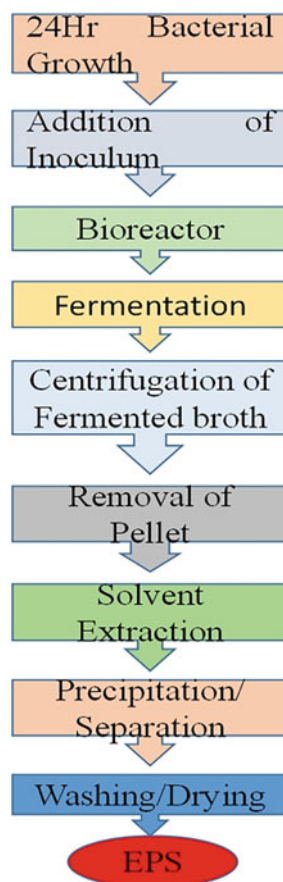
(continued)

Table 2 (continued)

Microorganism	Source of environment	Application	References
<i>Salipiger mucosus</i>	Spanish Mediterranean seaboard	Pseudoplastic, medicine, and cosmetic industry	Llamas et al. 2010
<i>Halomonas hydrothermalis</i> MB-45	Southern Ocean (Indian Sector)	Biofilm formation, stabilizing agent, viscosifying agent, and thickener	Gray and Biddlestone 1973
<i>Bacillus licheniformis</i> strain B3-15	Hydrothermal vent, Vulcano Island, Italy	Antiviral agent	Maugeri et al. 2002 ; Arena et al. 2006
<i>Pseudomonas</i> sp.	Southern Ocean (Indian Sector)	Biofilm formation, stabilizing agent, viscosifying agent, and thickener	Sengupta et al. 2019
<i>Halomonas maura</i>	Saltern at Asilah (Morocco)	Viscosifying agent, gelling agent, emulsifying agent, metal-binding activity	Bouchotroch et al. 2001
<i>Halomonas maura ventosae</i>	Saline soils in Jaén (south-eastern Spain)	Viscosifying agent, gelling agent, emulsifying agent, metal-binding activity	Martínez-Cánovas et al. 2004a
<i>Halomonas maura anticariensis</i>	Fuente de Piedra, a saline-wetland wild-fowl reserve in Malaga, southern Spain	Viscosifying agent, gelling agent, emulsifying agent, metal-binding activity	Martínez-Cánovas et al. 2004b
<i>Alteromonas hispanica</i>	Fuente de Piedra, south-east Spain	Viscosifying agent, gelling agent, emulsifying agent, metal-binding activity	Martínez-Checa et al. 2004
<i>Idiomarina ramblicola</i>	Inland hypersaline habitats in Spain	Viscosifying agent, gelling agent, emulsifying agent, metal-binding activity	Martínez-Cánovas et al. 2004c
<i>Idiomarina fontislapidosi</i>	Inland hypersaline habitats in Spain.	Viscosifying agent, gelling agent, emulsifying agent, metal-binding activity	Martínez-Cánovas et al. 2004c

EPS have a broad range of applications in various industries, such as food, pharmaceutical, and other industries. These biopolymers have various applications in that they are used as stabilizers, binding agents, gelling agents, lubricants, coagulants, film formers, and thickening agents (Sutherland [1997](#)). EPS are composed mainly of monosaccharides and several other noncarbohydrate substituents such as succinate and acetate. These EPS molecules are extremely pure, have regular structures, and possess unique rheological properties. Another characteristic feature of EPS that they are acidic or basic in nature. Dextran, produced by *Leuconostoc mesenteroides*,

Fig. 1 Flow chart of bacterial exopolysaccharides production



is used in plasma substitutes for shock and loss of blood (Silver et al. 1998). The lactic acid bacteria (LAB)-producing EPS, used as a stabilizer and thickener, also produced commercially (Singh and Das 2011). *Xanthomonas campestris* produces xanthan with a highly industrially valuable polymer. These bacterial EPS have wide industrial applications such as in the food, oil recovery, cosmetics, and toiletries industries. The fantastic rheological properties of this xanthan gum allows it to be used as stabilizing agent in suspensions and emulsions, and it is used in aqueous systems as a rheological control agent (Roberts 1996). Xanthan is also added to the uniformly suspended solid component in formulations used to improve the flowing ability of insecticides, herbicides, and fungicides in the agricultural field (De Angelis 2012).

Various studies put forward that EPS from *Spirulina* was composed of polysaccharides and used as anti-inflammatory and therapeutic agents. Spirulan, which is produced by *Arthrospira platensis*, is a sulphated polysaccharide, and has been

recognized to have the ability to inhibit pulmonary metastasis in humans. It is also involved in the prevention of adhesion and proliferation of cancer cells (De Morais et al. 2010). Similarly, the polysaccharide produced by the marine microbe *Vibrio diabolicus* is hyaluronic acid and is commercially known as the trade name 'Hyalurift.' These polysaccharides are important in bone integrity, and they show restoration activity (De Morais et al. 2010). Two bacterial EPS, GalactoPol, produced from *Pseudomonas oleovorans*, which is mainly composed of galactose (Freitas et al. 2009), and FucoPol, a fucose containing EPS synthesized from *Enterobacter* A47, were newly reported and have various applications. As a part of the investigation of new biopolymers with potential sources, several microbes are isolated from extreme environments, such as Antarctic ecosystems, deep sea hydrothermal vents, saline lakes and geothermal (Poli et al. 2010). Biofilm-producing *Micrococcus luteus* from the ship hull at Cochin, India, quantified and optimized EPS production. The higher production of EPS was at 56.7gm/L, at 96 h of incubation time, 27 °C incubation temperature, and EPS showing antimicrobial activities against the human pathogens (Nisha et al. 2020).

Exopolysaccharides from *Bifidobacterium bifidum* WBIN03 and *Lactobacillus plantarum* R315 showing antimicrobial activity against *Cronobacter sakazakii*, *E. coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Candida albicans*, *Salmonella typhimurium*, and *Shigella sonnei* were reported by Li et al. (2014). Crude EPS from *Lentinus subnudus* was tested for its antibacterial activity in Swiss albino rats infected with *E. coli* and *Pseudomonas aeruginosa* and it was found that EPS possess antibacterial activity (Majolagbe et al. 2013). Mahendran et al. (2013) reported similar study on potential antibacterial effects of EPS from *Ganoderma lucidum* and *Lysinibacillus fusiformis*. Rajasree et al. (2014) studied EPS from marine *Exiguobacterium* spp., and results showed inhibition activity on *Alteromonas* spp. and *Gallionella* spp.

Broadly characterized, studied, and commercially profitable EPS xanthan gum, which is an initially produced biopolymer (Rosalam and England 2006) from a microbial polymer produced by *Xanthomonas* spp., is a most extensively studied and widely accepted commercial microbial polymer. Gellan, rhamsan, welan, and diutan are EPS that are distinguished by differences in composition and side chain linkage. Alginate is a polymer secreted by the genera *Pseudomonas* and *Azotobacter* and is a linear polysaccharide that is composed of guluronic acids and mannuronic acids and forms mixed and poly-mannuronic acid sequences. Alginates from bacteria and algae show differences in acetylated alginates (Rehm 2009). Glucans – glucose homopolysaccharides – vary in glycosidic linkage, chain length, degree and type of branching, confirmation of polymer, and a molecular mass. There are two types of glucans, β -glucan and curdlan and α -glucans. Several microbes from the genera *Gluconacetobacter*, *Agrobacterium*, *Achromobacter*, *Aerobacter*, *Rhizobium*, *Sarcina*, and *Salmonella* are capable of producing cellulose (Chawla et al. 2009). α -Glucans is an extracellular enzyme produced by dextranucrase from sucrose, by numerous bacteria of the genera *Leuconostoc*, *Streptococcus* and *Lactobacillus*. Hyaluron from respective bacterial strains such as *Pseudomonas aeruginosa* and *Streptococcus* A and B attenuated strains (Rehm 2009), is composed of glucuronic

acid and a linear polymer composed of a repeating disaccharide. N-acetylglucosamine is a linear polymer with repeating disaccharide units (Ruffing and Chen 2006). Microbial succinoglycan – are secreted by some soil bacterial species such as *Rhizobium*, *Alcaligenes*, *Pseudomonas*, and *Agrobacterium* (Glenn et al. 2007). It is a branched EPS with a galactose and glucose backbone and the side chain tetrasaccharide composed of glucose residues. Levan is an extracellular enzyme produced from diverse bacterial species such as *Bacillus*, *Rahnella*, *Aerobacter*, *Erwinia*, *Streptococcus*, *Pseudomonas*, and *Zymomonas*, and is a highly branched fructose homopolysaccharide synthesized by an enzyme levan sucrose from sucrose.

Extracellular polymeric substances/exopolysaccharides are accountable for the adhesiveness of biofilm on the biotic/abiotic solid surfaces, the cohesion of microorganisms, influencing spatial organization, acting as adhesive in cells, allowing interactions among microorganisms, and some of the resistant actions, etc. Such biological functions are essential for formation and other activities. The EPS /biopolymers stabilize the bacterial aggregation via various interactions among macromolecules, such as dispersion forces, electrostatic interactions, and hydrogen bonds. The outer secretion of a jelly like 3D structure in the microbes allows them to develop a strong consortium (Flemming et al. 2000). EPS produced from *Sphingomonas paucimobilis* have properties such as surface activity, which promotes and influences adhesion through secretion of an extracellular polymeric substance (Azeredo and Oliveira 2000). Some of the protein adhesins play a key role in the attachment of microbes to the surfaces. The EPS produced from *Caulobacter crescentus*, known as holdfast, is mainly influenced by the initial surface attachment together with other cellular structures (Wan et al. 2013). The

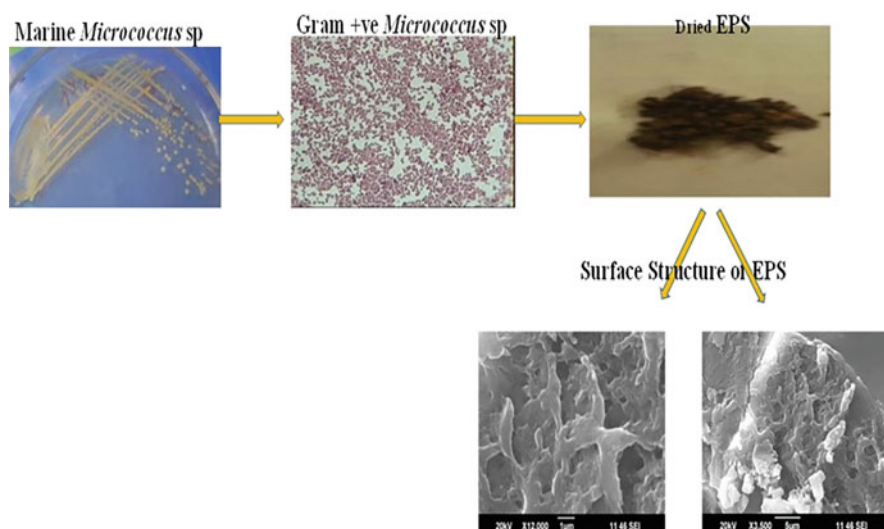


Fig. 2 Exopolysaccharides production by marine *Micrococcus luteus*

higher production of EPS by microbes can influence the cell adhesions. Large quantities of EPS enhance the attachment via interaction in between the functional groups such as uronic acid and acetyl groups; this statement has been confirmed by evaluating 27 bacterial species. The process of the interactions between the EPS and functional groups is unknown and the matrix formed by EPS can influence the chemical communication (Flemming et al. 2007).

In a few of bacterial cells, ex-DNA seems to be answerable for the adhesion capabilities of EPS rather than the presence of polysaccharide. The structural and functional activities of ex-DNA studies have not been reported entirely, whereas a few works show the responsibility of the structural properties and cohesion of EPS involved in the process of adhesion onto the solid surfaces and signaling. In the biofilm formation of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Ralstonia solanacearum* ex-DNA is an important structural component. Active secretion of EPS by the microorganisms (Whitchurch et al. 2002) however, it is not essential in the formation of biofilm by *Streptococcus epidermidis* (Flemming and Wingender 2010). This statement is dependent upon the treatment with DNA-ase1, which inhibits the formation of biofilm by *S. aureus* but not *S. epidermidis* (Izano et al. 2007).

Ex-DNA are very important in the components of biofilms produced by different microbes, these biofilm microbes are surrounded by an EPS. There is a much less detailed understanding on this area because less research has been done. In bacterial cells, the rate of transformation and the rate of conjugation are higher in biofilms. *Campylobacter jejuni* transfer antibiotic-resistant genes by transformation and show a higher rate in the biofilm than they do in planktonic cells (Bae et al. 2014). Related studies reported that the age of biofilm and the concentration of DNA influence the frequent rate of transformations, whereas the higher density of planktonic cells inhibits the transformation in biofilm. Moreover, the number of events used to observe can depend on the techniques, which are used here to detect the conjugative gene transfer in a biofilm. 100-fold higher rates of conjugation were detected by the use of confocal laser scanning microscopy. It is higher than when plating techniques are used (Hausner and Wuerztz 1999). Some of the research discussed and suggested on ex-DNA can be used as an alternative to bioactivity measurements in environmental studies. The fraction separation of ex-DNA and the evaluation/analysis in the case of complex samples such as soils have not been developed.

Studies on polymers reported that, surface active agents are amphiphilic substances that are used in various sectors in the modern industry, most of these are synthesized by organochemical synthesis. Biosurfactants and bioemulsifiers are produced by the biological sources and have gained more interest in the past decades owing to the structural and functional diversity. These compounds are mainly of high biodegradability, and less toxic when compared with chemically synthesized compounds. Research reviews on this substance give an overall summing-up of the classification of various types of biomolecules with surface active abilities, and more of these molecules are awaiting discovery, some of them with production on an industrial scale. The marine environment is a mostly unexploited source of novel surface-active agents. Microbes from marine sources, mainly bacteria that are living

in association with microalgae are potential sources of surface-active agents with various biotechnological applications. In the production of a surface-active agent from a different microbe, the yield can be too low to meet the quantity demanded by the industries. This is major problem remains an important part of their future commercial development.

Applications of Biopolymers

Polymers from microbial sources have unique properties with abundant materials and applications in various sectors.

Industrial Applications

Microbial polymers are mainly used as industry standard material, in combined with other materials to enhance their desired properties and applications. These polymers are widely used in packaging in various sectors, PHA, polylactic acid and starch being easily available, water resistant, and inexpensive. Microbial polymers are used as a biocement during concrete preparations for industrial construction and interior decoration. Some of these biopolymers are able to remove metals from water and is beneficial in waste water treatment. Biopolymers are used in the automotive industry as a component for making interior and exterior parts, engines, exhausts, steering wheels, electrical components, etc. These polymers are also used in food industries as thickening agents, gelling agents, and stabilizers.

Biomedical Applications

Most of the biopolymers are widely used in the biomedical field and are nontoxic, degradable, biocompatible, and can be applied in drug delivery, tissue engineering, and the pharmaceutical industries. They are very porous and are used as wound healers, for example. Microbial polymers/polypeptides are easily available and inexpensive materials; therefore, they increasingly used as biomedical materials. Many studies reported the ortho-dental applications of bacterial polymers, hydrogels, etc., and their suitability. Biocompatible polymeric substances including polysaccharides, polyamides, polyesters, and polyphosphates have diverse applications such as antioxidant, antibacterial, antifungal, and antitumor wound healing.

Dextran is a potential drug carrier polymer, with targeting approaches in several organ therapies, such as colon-centered therapies and other intestinal/alimentary tract therapies, owing to its degradability. Liver-targeted drug therapies are also successfully completed with dextran-based nanomaterials.

Gellan and its derivatives have wide applications such as tissue engineering, gene therapy, protein carriers, regenerative medicine, and drug delivery. Gellan is mainly

used as a gel or capsule coating, which helps to release the drugs with a modified profile. These are used against gastric and peptic ulcers, inflammation, arthritis, etc. Gellan gum beads are used against diabetes as hypoglycemic agents. Gellan can be fabricated/modified into fibers, films, and 3D structures.

Curdlan is a potential wound-healing agent with reduced pain, speedy healing, and lowering of the number of dressings of wounds used when compared with the standard treatments.

Polyhydroxyalkanoates have been mainly used in soft-tissue engineering, such as heart valves, tendons, nerves, and blood vessels, and are also used in life-saving areas such as cardiac muscle regeneration, successfully mimicking myocardial muscle activity. These PHAs are used to encapsulate a drug for controlled drug delivery.

Cosmetic Applications

Biopolymers can be used as cleaning agents for pedicures and manicures and are used as the natural ingredient for hair care products, sunscreen lotion/creams, and in beauty creams/lotions.

Nanotechnology

Biopolymers are biomaterials used in the production of nanomaterials and are in higher demand in green chemistry. Green synthesis of various nanomaterials constitutes a new trend in research on nanotechnology.

Agriculture/Fishery

Bacterial EPS play an important role in symbiotic relation between nitrogen-fixing bacteria such as *Rhizobium* and the leguminous plants. *Sinorhizobium meliloti* is able to produce a polymer, succinoglycan. Some of the studies reported that the plants are capable of identifying the structure of rhizobial EPS and the expressions of the receptors. Biopolymers can be utilized for making fishing nets, beehives, fishing traps, and fertilizers.

Other Applications

Biopolymers are used to produce footballs and other hollow balls, golf and badminton equipment, etc. These materials are also used for the manufacturing of insulated wires, cables, electronic devices, printed circuit boards, etc.

Conclusion

Many studies are continuing to explore alternatives to petroleum-based products that would be biodegradable or renewable to reduce the risk of pollution. Biopolymers are the best solution to the problem; hence there are so many challenges such as the limited rate of production, cost of production, and sustainability. Prokaryotes, especially bacteria, are the superior cell factories that have the ability to convert carbon and nitrogen sources into various intracellular and extracellular biopolymers, such as polysaccharides, polyphosphates, polyamides, and polyesters. Microbial polysaccharides are renewable sources for the formation of hydrocolloids and can be used in the food industries, pharmaceutical industries, and in various other industrial applications, e.g., xanthan, dextran, alginate, and gellan. In the case of microbial polymers, only few of these are commercialized. Microbial biopolymers play an important role in their pathogenicity/virulence. These microbial biopolymers have diverse characteristics and properties, and are suitable for medical and industrial applications.

Cross-References

- [Biopolymers](#)
- [Biopolymers from Renewable Sources](#)
- [Material Applications of Gelatin](#)
- [Nature-Inspired Biomimetic Polymeric Materials and Their Applications](#)
- [Peptide-Based Biopolymers in Biomedicine and Biotechnology](#)

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Biomolecules from Humus Oil

6

Samuel Ogbeide Ebhodaghe

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Abstract

Humic substances are complex biopolymers found in soils, water bodies, and sediments. The application of humus-based materials in biomedicine is emerging because of its medicinal properties. Specifically, humic acid could demonstrate antiviral action against several viruses. This chapter summarizes recent advances on the effect of several interventions on humus composition, properties, and structures. It also highlights the biomedical applications of humic substances, especially in drug delivery.

Keywords

Humus · Humic acids · Biomolecules · Polymers · Biomedical applications

S. O. Ebhodaghe (✉)

Chemical Engineering Department, University of Benin, Benin City, Edo State, Nigeria

e-mail: samuel.ebhodaghe@eng.uniben.edu

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Introduction

Humus is a common organic compound present in the environment. It occurs in concentrations between 1 mg(C)/L to over 100 mg(C)/L in water bodies (Maier 2015). It is also found in soils, water, and sediments since it can be obtained from plants, algal, and microorganisms (Pandey et al. 2000) when it undergoes physical, chemical, and biochemical changes. In brief, humus is got from decomposing plants and biomasses. As such, it is considered ubiquitous and heterogeneous because of several reasons, including formation mechanisms and processes, as illustrated in Fig. 1.

Recent advances have examined the effects of several interventions on humus composition, properties, and structural characteristics in different locations under varying environmental conditions. First, research on identifying humic substances based on a nonconventional transformational process was investigated. Bezuglova and coworkers (Bezuglova and Komarov 2022) identified humus through its unique characteristics during peat's conversion. They studied this conversion process based on some growing seedlings for about 5 years. The conversion processes were identified as hydrolysis of organic products, initial transformation process, and humidification of the lignin structures. Building on this, Ndzelu et al. (2021) examined the effect of tillage on the composition of soil humus and humic acid structural characteristics in soil. The study showed that avoiding soil tillage was a better alternative to tillage in enhancing the sequestration and stability of soil carbon. Similarly, Raiesi (2021) reported on the effects of wheat dry farming as well as abandonment of the same on the quantity and quality of humic substances in an ecosystem. These factors were used to determine the “soil degradation and

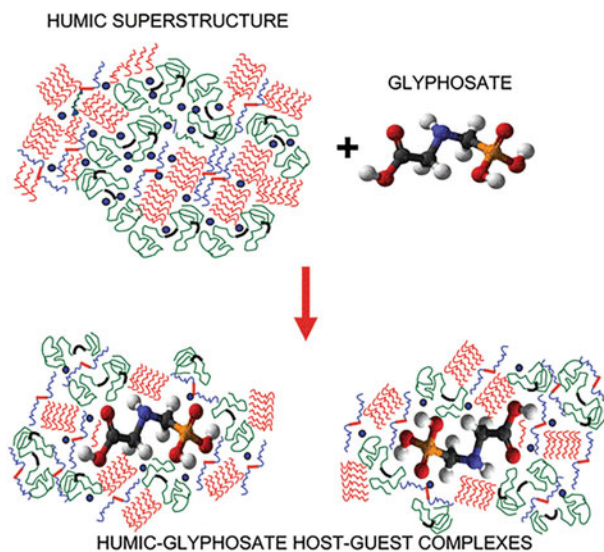


Fig. 1 Illustration of humus formation mechanisms. (Reproduced with permission from Mazzei and Piccolo (2012))

ecological restoration in semi-humid pastures.” As such, land-use changes were shown to affect soil organic matter. Depending on the location, the (bio) chemistry of soil humus and humic substances could differ (Baveye and Wander 2019). In Kuban rice crops, Sheudzhen and Gutorova (Sheudzhen and Gutorova 2021) investigated the influence of liquid manure on the humus state of gleyic and chernozem soil grown with rice. The study reports increased soil humic acid in the region of Krasnoarmeysky district of Krasnodar territory. Another recent study (Levshina and Matyushkina 2021) presented different concentration distributions of dissolved aluminum, iron, copper, chromium, vanadium, nickel, and zinc, and their humus complexes in Zeya reservoir water. When compared to iron, the latter had the highest concentration distribution. Similarly, few studies have also reported properties of humic substances in Eastern Siberia (Milkheev and Tsybenov 2021), Uzbekistan (Kamilov et al. 2021), and others (Sarapulova 2021).

Humus is a complex polymer formed from polymerization reaction of several monomers that are considered decomposing organic products, which can be repolymerized, as shown in Fig. 2. Therefore, humus undergoes a dynamic equilibrium state in nature (Selyanina et al. 2012). This is also because the humus content of several soils hardly changes, which makes the rate of formation similar to the turnover (Varga et al. 2008). This turnover (2–5% per annum) is basically a function of climate changes (Yuan and Chen 2010). Though biomolecules could be obtained from different sources, we have focused on soil and microalgae-based biomolecules. Furthermore, the chemical properties and biomedical applications of the same have been presented. In conclusion, we have examined recent advances in polysaccharide-based naturally occurring biomolecules.

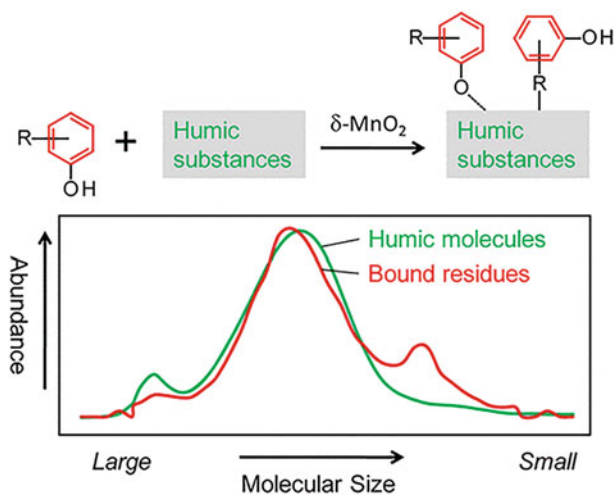


Fig. 2 Polymerization reaction of soil humic substances. (Reproduced with permission from Li et al. (2012))

Table 1 Overview of fungicides in soils, with their chemical names

Fungicides	Chemical names
Azoxystrobin	Methyl (E)-2-[2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]-3-methoxyacrylate
Cyprodinil	4-Cyclopropyl-6-methyl- <i>N</i> -phenylpyrimidin-2-amine
Fludioxonil	4-(2,2-Difluoro-1,3-benzodioxol-4-yl)th-pyrrole-3-carbonitrile
Mancozeb	Zinc manganese ethylenebisdithiocarbamate
Metalaxyl	Methyl <i>N</i> -(methoxyacetyl)- <i>N</i> -(2,6-xylyl)-DL-alaninate
Penconazole	(RS)-1-[2-(2,4-Dichlorophenyl)pentyl]-1H-1,2,4 triazole
Procymidone	<i>N</i> -(3,5-Dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide
Tebuconazole	(RS)-1- <i>p</i> -Chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4,-triazol-1-yl-methyl) pentan-3-ol
Vinclozolin	(RS)-3-(3,5-Dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione
Propiconazole	(2RS,4RS; 2RS,4RS)-1-[2-(2,4-Dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1H-1,2,4-triazole
Pyrimethanil	<i>N</i> -(4,6-Dimethylpyrimidin-2-yl) aniline
Quinoxifen	5,7-Dichloro-4-quinolyl-4-fluorophenyl ether

Toxicology: Fungicides in Vineyard Soils

Ensuring toxic-free soils is the desired goal in several agricultural regions. However, the presence of pesticides in soils raises serious concerns. This is because studies have shown that numerous dangers and risks are associated with their extensive usage in soils. So, the sorption of pesticides is an important process that could be affected by their solubility in soils. Generally, several fungicides are slightly soluble at a temperature range of 20–30 °C. For example, azoxystrobin, cyprodinil, fludioxonil, mancozeb, penconazole, procymidone, tebuconazole, and vinclozolin have solubility ranging between 1 and 100 mg/L, while quinoxifen is exceptionally insoluble with less than 1 mg/L. At the same temperature range of 20–30 °C, metalaxyl, propiconazole, and pyrimethanil have a notably higher solubility of 100–10,000 mg/L (Kamrin 1997). The chemical names of these specific fungicides are shown in Table 1.

Several studies have reported that the increasing “use of pesticides in areas of wet subtropical climate” causes a high incidence of copper concentration. Thus, copper-based pesticides are also vineyard soil contaminants. In the upper layers, Brazilian soils have one of the highest concentrations of copper contents ranging from 37 to 3216 mgCu/kg (Casali et al. 2008; Mirlean et al. 2007), according to the findings. Depending on the depth, Bulgarian soils have been shown to contain least copper contents in their upper layers, while Spanish soils could be also least in increased depths.

Properties and Composition of Humic Substances

Humic substances can be characterized by their color, total exchangeable acidity, alpha amino nitrogen, nitrogen, methoxyl, vanillin, and carboxyl and phenolic contents (Rashad et al. 2022; Lawrence 2017). Humic substances are usually black

in color, with low methoxyl and nitrogen contents of about 3–6% for the latter. Alternatively, humic substances could contain high carboxyl and phenolic hydroxyl contents, with over 150 cmo/kg total exchangeable acidity. Specifically, humic substances consist of lignin, proteins, and humic and fulvic acids. As per the basic composition, lignin contains 62–69% carbon, 5.0–6.5% hydrogen, and 26–33% oxygen, with no nitrogen content. Similarly, humic acids are composed of 52–62% carbon, 3.0–5.5% hydrogen, 3.5–5% nitrogen, and 30–35% oxygen. Also, proteins contain same carbon, hydrogen, oxygen, and nitrogen in proportions of 50–55%, 6.5–7.3%, 19–24%, and 15–19%, respectively.

Humic substances are composed of peat, sapropel, oxifulvic acid, potassium humate, and carbohydrate-derived fulvic acid. Peat is a biomass mainly made of organic matter (about 65%) and inorganic matter (about 35%). They mostly occur in wetland regions (Bujang et al. 2011). Properties of peat include moisture content, bulk density, specific gravity, void ratio, permeability, compressibility, and shear strength. These properties represent “the engineering behavior of peat,” which can be enhanced using fiber reinforcement, sodium silicate grout system, and others (Sina et al. 2011).

Sapropels are products of accumulated biomass materials, usually located under freshwater bodies (Bogush et al. 2022; Ivanova et al. 1997). Peat, as well as sapropels and other humic substances, are basically used in the treatment of dermatitis (Wolina 2009), psoriasis (Codish et al. 2005), rheumatoid arthritis (Gungen et al. 2012), and wounds (Vysokogorskii et al. 2009).

Studies have been conducted on the size of the humic substances. For instance, Guo et al. (2016) report HS from dairy manure and sugarcane pressmud compost as 300–600 nm. Calvo et al. (2016) also have noted that HS could demonstrate a molecular weight of 500–1000,000 Da due to their hydrophobic interactions and hydrogen bonding with heterogeneous polymers (Liu et al. 2011; Canellas et al. 2015).

Applications/Prospects of Humic Substances in Pharmaceutical Research

When humic substances are dissolved, they produce humic acid. Humic acids possess desirable medicinal properties of antiviral action, anti-inflammation, and others (Yamada et al. 1998), which demonstrate their usefulness in biomedical applications. Some of these areas of biomedical applications include the treatment of ulcers and herpes virus infection (Mishra et al. 2015). However, humic substances could also be used to detoxify toxic sites (such as metal-polluted lakes (Petit 2004; Tan 1994), sequest metals (Pandey et al. 1999), enhance crop production (Mishra et al. 2015), extract organic substances from water (Biggs et al. 1985), energy provision (Talmage et al. 2007), and cleaning up of nuclear power plants (Sollins et al. 1996)).

Humic substances could play a significant role in cancer treatment due to their “biochemical and molecular effects.” This is because the emerging role of molecular

simulations involving humic substances could guide new experimental designs, as well as “predicting properties and phenomena at the molecular scale” (Jacob et al. 2019). Other areas include their role in cancer etiology.

Conclusion

Humic substances have unique structures and composition, with dynamic formation and turnover rates. The main advantage of humus materials is that they can be obtained from different sources, unlike polysaccharides. Since only 20% of humus in certain soils occur as lipids, more research is needed to investigate the role of ecological restoration in enhancing the yield. The effects of crop dry farming and abandonment on the humic composition should be investigated.

Cross-References

► [Mechanical Properties of Biopolymers](#)

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Soy Protein Biopolymer

7

B. S. Subi and S. Shabanamol

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B. S. Subi (✉)

Indira Gandhi College of Arts and Science, Kothamangalam, Kerala, India

e-mail: subibs@igcas.org

S. Shabanamol

SAFI Institute of Advanced Study, Malappuram, Kerala, India

e-mail: bioshabana@siasindia.org

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Abstract

The use of polymers based on biological materials, rather than those that are based on petroleum, to create ecofriendly biologically degradable materials has gained popularity in recent years. Since they are less expensive and easier to produce than natural polymers, synthetic polymers are widely used. But they cause environmental contamination and are not biodegradable. Therefore, scientists advised choosing polymers that are naturally eco-friendly. This idea explains why soy protein, a cheap, easily accessible, and biodegradable ingredient, is so popular. It has superior biocompatibility, biodegradability, and better processing qualities and is 90% protein. Another appealing aspect of it is its use in numerous industrial fields. The aim of this chapter is focused on discussion of history of soy protein, composition, physicochemical properties, abiotic factors involved in improving the mechanical properties of soy protein, soy product production methods, and bio-nanocomposites. Moreover, some applications of soy protein polymer are also discussed.

Keywords

Biopolymer · Bio-nanocomposite · Soybean · Soy protein concentrate

Introduction

Biopolymers are natural polymers produced by or made from living things. They can be produced by chemical process from biological materials or can be synthesized solely from living organisms. Among the earliest modern biomaterials manufactured from natural biopolymers were rubber, linoleum, celluloid, and cellophane. Cellulose, the most common organic substance and most abundant biopolymer on the earth, is used to make celluloid and cellophane (Hassan et al. 2019). Various plants, including maize and soybeans, as well as different species of trees and even bacteria, may be the source of this resource. Concerns over the damage that pollution, from fossil fuels and petrochemical wastes which are the natural and traditional source of biopolymers, has on the environment are increasing. Several researches have been conducted to investigate various other alternatives to petroleum-based goods that are renewable and biodegradable, resulting in a lower risk of environmental damage. Since biopolymers are typically biodegradable materials produced from renewable basic resources, the possibilities of new product derivation are the hot topic in various fields like

health, pharmaceuticals, nanotechnology, food industries, enzyme kinetics, etc. Following are major categories for biopolymers:

1. Classification based on monomeric unit used and the structure of the biopolymers formed (Reddy and Yang 2011)
 - Long polymers of nucleotides like DNA and RNA with at least 13 or more polymers of amino acids
 - Polymers of carbohydrates with linear bonds
2. Classification according to their origin (Tansaz and Boccaccini 2016)
 - Polyesters: polyhydroxyalkanoates, poly lactic acid
 - Proteins: silk, collagen/gelatin, elastin, rexlin, adhesic, and polymonoacid; soy, zein, wheat, gluten, casein, and serum album
 - Polysaccharides (bacterial): xanthan, polygalactosamine, dextran, gellan, and cellulose
 - Polysaccharides (fungal): pullulan, chitin, and yeast glucan
 - Polysaccharides (plant/algal): starch, cellulose, agar, alginate, carageenan, pectin, konjan, and various gums
 - Polysaccharides (animal): chitin, hyaluronic acid
 - Lipids/surfactants: acetoglycerides, waxes, and emulsion
 - Polyphenols: lignin, tannins, and humic acid
3. Classification based on the type of origin
 - Synthetic biopolymers are biodegradable polymers made from renewable materials like polylactic acid.
 - Natural biopolymers are biopolymers produced spontaneously by living organisms.
 - Microbial polymers: produced by microorganisms such as bacteria, fungi, and algae.

Biopolymers can either be categorized as thermoplastics, thermosets, or elastomers based on how they react to heat. Biobased thermoset biopolymers have recently outpaced biobased thermoplastic biopolymers in volume (Post et al. 2020). Likewise, one more classification is based on their combination as blends, composites, or laminator. Blends of biopolymers include a mixture of polymer-like ElovioR (BASE.AG) which is said to be a mixture of PLA and PBAT. Biocomposites are biopolymers which are synthetically polymer strengthened using fibers of natural origin such as hemp, flax, banana, wood, jute, number of grass-like materials and flax, hemp, jute, banana, wood, various grasses, and condiments (Rajeswari et al. 2021).

It is advantageous to have a biodegradable plastic that undergoes microbial decomposition in the soil for numerous biomedical, agricultural, and ecological purposes. Many scientific investigations and efforts have been made to improve plastics' biodegradability. However, their efforts have been focused mostly on the following areas. Biodegradable polymers, synthetic polymer modification, and biodegradable polymer composites are all examples of new biodegradable polymers. Biopolymers as starting materials have received a lot of interest in the search

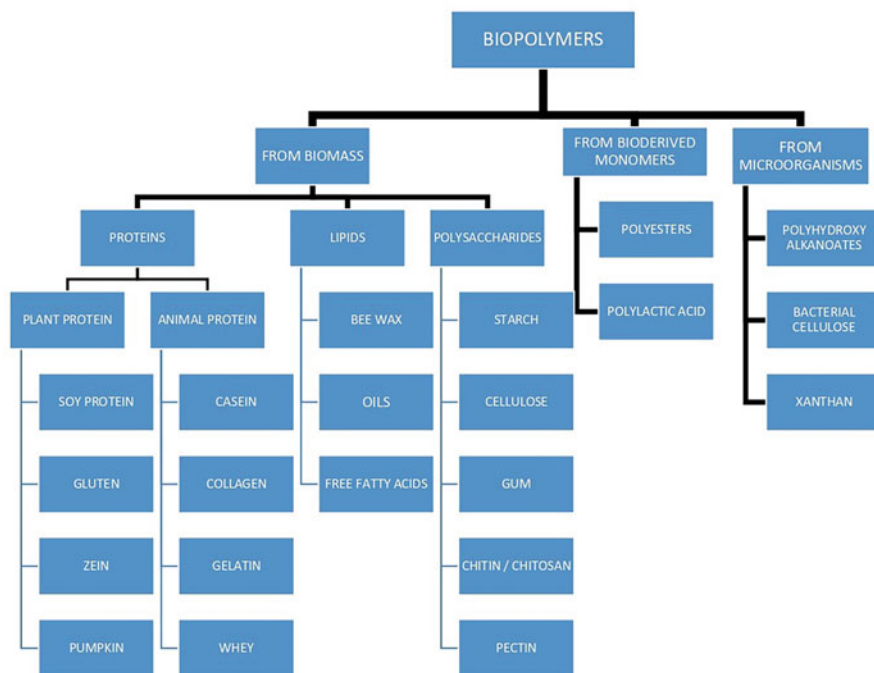
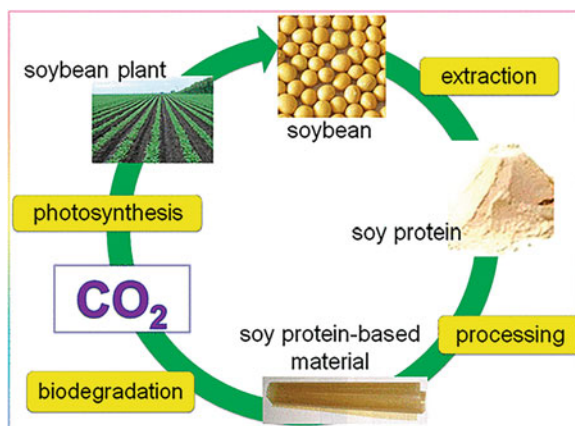


Fig. 1 Classification and components of biopolymers

for biodegradable polymers made from renewable resources. Green composites are a particular type of biocomposite that are reinforced with natural fibers using a biobased polymer matrix. We can rid our environment of plastic waste by substituting green composite items for synthetic plastic ones. Biobased polymers have played a significant part in the evolution of the plastics sector today. Biopolymers have been utilized by humans for food, clothing, and furniture throughout history. Among these, soy protein has gained much scientific interest for being developed as ecofriendly protein material with efficient properties like biodegradability, biocompatibility, regeneration, etc. (Echeverría et al. 2014). Plant proteins are polymers that are biodegradable as well as renewable. Soy protein is used as starting material for making plastics (Fig. 1). It is also used as filler or expender to reduce the price of plastics originated from petroleum products to assure the eco-friendly biodegradability. Apart from these characteristics, it is used as food ingredient, and also it has attracted great attention in nonfood application as polymeric materials. Composites made of soy protein were frequently utilized for hard packaging, transportation, and housing. Green composites were created using soy protein resins for a variety of uses, including glues or adhesives, plastic films, coverings or coats, emulsifiers, and blended hydrogels. They are also being considered to be substances which have immense use in biotechnology and medicine (Reddy and Yang 2011) (Figs. 2 and 3).

Fig. 2 Diagrammatic representation showing stages of producing soy products from young soya plants (Song et al. 2011)



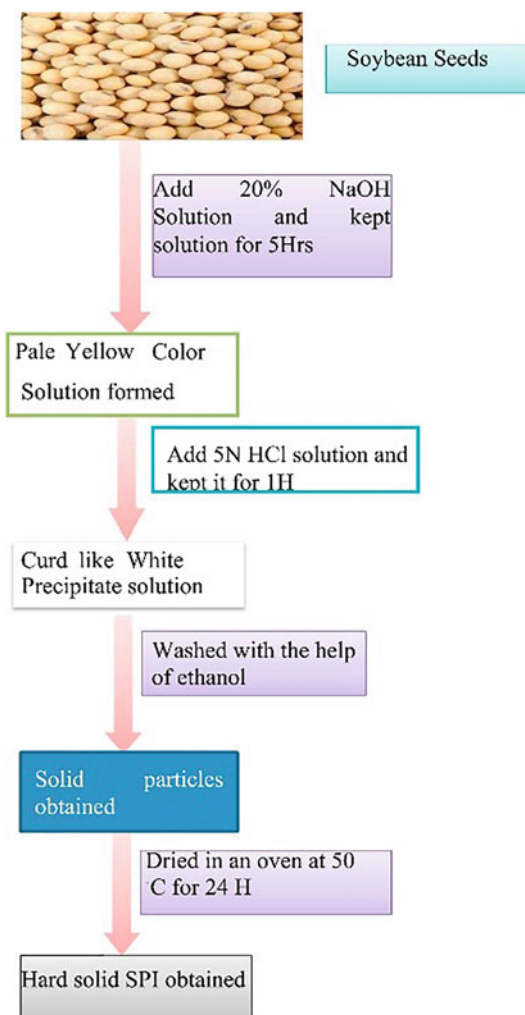
History of Soy Protein (Dupare et al. 2008)

See Table 1.

Composition of Soy Protein

Soyproteins (SP) are extracted from soybeans. It belongs to the plant species *Glycine max*, family Fabacea. They contain ~38% protein, ~30% of carbohydrates, ~14% moisture and minerals, and ~18% oil (Tansaz et al. 2016). It is a globular protein. The main components of soy protein are glycinin and β -conglycinin. Their content, rates, and biosynthetic dynamics vary depending on the type of soybean and the environment in which they live. The molecular weight of glycinin and conglycinin is between 300–400 KDa and 150–250 KDa. β -conglycinin is a trimer or a hexamer when in solution, while glycinin is a hexamer (Hammond et al. 2003). Glycinin is a protein having quaternary structure that is stabilized by disulfide, electrostatic, and hydrophilic interaction. 6 A-SS-B subunits are made up of polymers of acidic and basic proteins bonded by di sulfide linkages. The subunits of the same are found to be arranged as double hexagon stacks and result in a shape of hollow horizontal cylinder. Glycinin has acidic or basic polypeptides linked by disulfide bond. Inner portions of the glycinin molecule are rich in proteins containing basic amino acids. Meanwhile, beta conglycinin forms important portion of 7S and is having 150–180 KDa which contains 3 sub units such as α , α' and β . They are linked through hydrophobic interactions. They interact to produce 7 isomers. Molecular weight of these subunits is 72 KDa, 68 KDa, and 52 KDa. The N-terminal aspartic acid residue of the original β conglycinin is glycoprotein consisting of carbohydrates linked as a single unit. Per protein molecule, the carbohydrate moiety consists of 12 glucosamine residues and 38 mannose residues. So their structure is different, and that

Fig. 3 Extractions of soy protein isolate (Gautam et al. 2021)



reflects in their physicochemical properties too. The isoelectric point of conglycinin is 4.8, and that of glycinin is 6.4. The amino acid property in soya protein is like 18% basic and 20% acidic in nature (Gorissen et al. 2018). The amino acid content in soy protein is given in Table 1. It divides into fractions 2S, 7S, 11S, or 15S depending on the molecular weight and sedimentation coefficient. Of the soy proteins, the 7S and 11S globulins account for 37% and 21%, respectively. Glycinin serves as a gelling, emulsifying, and foaming agent when combined with other film-forming proteins. In comparison to glycinin, β -conglycinin is less heat stable and denatures between 70 °C and 80 °C (Table 2).

Table 1 The timeline of commercialization of soy protein

Time period	Contributions
700 BC	First written record of soybean cultivation record of soybean cultivation for China
1712	Soybean included in Europe
1804	Soybean was introduced to the USA from Asia/China
1900	Research on agricultural sources for plastic production including soy protein
1913	Britain and France bagged patents for generating bioplastics from soy protein
1917	First US patent for soy protein
1933	As a result of Henry Ford's soy bean experiment, it was discovered that soy protein could be shaped into miniature plastic car parts and that soybean oil produced outstanding paint for painting automobiles and binding molds
1936–42	Regional Soybean Industrial Products Laboratory, Urbana, published a continual series of studies on its initiative to produce polymers from soy protein and meal
1942	150 million lbs. of soy-meal plastic was produced which was a derivative of phenol-formaldehyde, soy, and wood flour
1960s	Although the worldwide soya bean market has declined, Japan's booming livestock sector has begun to employ soy protein meal as a protein and energy source
1970s	Soybean consumption in Europe increased gradually
1976–2005	Soybean plantings increased by 50% in the USA, while national average soybean yields increased by about the same amount
1980s	In Europe, demand for soy meal and soy oil fueled expansion of processing capacity
1990s	Demand for soybean products spread to China, South Korea, Mexico, Indonesia, Turkey, and Philippines
2000s	China's overall capacity for processing soy beans has quadrupled

Types of Soya Protein Product

Soy protein products are mainly produced in 3 forms. They are flour, concentrate, and isolates of soy protein. The major difference among the above components is in the content of proteins, in which SPI has uppermost and soy protein flour has the lower most percentage of protein. The carbohydrate content is high in soy protein flour. At the time of production of soy protein concentrate, the soluble carbohydrates are removed leaving the insoluble types. But during soy protein isolate production, both soluble and insoluble carbohydrates were removed.

Soy Flour

Soy flour contains about 56% protein. The fraction is like 11S fraction and 7S fraction; each contains ~35% of the protein. Storage globulins, glycinin and conglycinin, are the major components of the two fractions. The native glycinin contains tightly folded polypeptides linked by disulfide bonds. Soy protein flour is

Table 2 Amino acid content in soya protein concentrates

Sl. No.	Amino acid	Soya protein concentrate (mg/100 g)	% of total amino acids
1.	Alanine	2677	4.3
2.	Arginine	4642	7.4
3.	Aspartic acid	7249	11.5
4.	Cysteine	886	1.4
5.	Glutamic acid	12,013	19.1
6.	Glycine	2688	4.3
7.	Histidine	1578	2.5
8.	Isoleucine	2942	4.7
9.	Leucine	4917	7.8
10.	Lysine	3929	6.2
11.	Methionine	814	1.3
12.	Phenyl alanine	3278	5.2
13.	Proline	3298	5.2
14.	Serine	3369	5.4
15.	Threonine	2474	3.9
16.	Tryptophan	835	1.3
17.	Tyrosine	2301	3.7
18.	Valine	3064	4.9
19.	Total amino acids	62,954	100
20.	Total EAA % total	23,832	37.9
21.	Total BC AAA % total	10,923	17.4

produced using defatted and fine ground shavings or flakes and meals after passing over meshed screen of standard sizes of 100. For manufacturing flakes with lesser fat content, clean, dry soya beans are subjected to deshelling and finally cracked. These cracked beans will be heating at 74 °C and flaked. The most widely used method is solvent extraction using hexane for extracting oil. About 1% or less amount of oil is reduced, and the solvent solubilizes the soy bean fat content. Later, the organic content in the flake product will be removed. A flash solventizing system can be used for the production of edible and industrial soy protein (Vavlitis and Milligan 1993). This method provides mild heating condition, minimum retention time to minimize protein denaturation, and low moisture condition. Finally, high soluble and nonfat containing de oiled flakes called white flakes. Soy flours are available in three forms like highly dispersible soy flour and in between these two forms of soy flour type.

Soy Protein Concentrate

Soy protein concentrate is a form of soy protein product. It contains about 70% protein. These are usually produced by separating majority of nonproteinaceous components in the soy flakes having high protein solubility which were subjected for

previous dehushing and fat-removal processing. This contains mainly soluble carbohydrates and some low molecular nitrogenous substances and minerals. The amino acid composition of soy protein concentrate differs somewhat from the original soy flour. Commonly, three methods exist for manufacturing soy protein concentrate. Each process produces a different type of concentrate with different properties. These processes include aqueous-alcohol wash, aqueous extraction combined with thermal denaturation and acid wash. The first two methods result in the denaturation of the final soy protein product; in contrast, acid wash will not denature soy proteins. pH of the dispersed water will be adjusted to an acidic range (4.2–4.5) using HCl in order to dissolve the soluble sugars. Hence this will help not to go for other specific solvents. Concentrate of solids is usually neutralized and then spray dried.

Soy-Protein Isolate

This is one of the popularly available soy-protein product containing 90% of proteins and is usually produced by utilizing the property of isoelectric point. Soy flakes which were subjected to fat content removal will be mixed with warm water at a pH of 7.5 to 9. By centrifugation, the soluble proteins and carbohydrates were partially recovered from the insoluble fractions. In order to precipitate the majority of the proteins, HCl is added to the solution, lowering the pH to 4.2–4.5. The precipitated proteins are rinsed clean of the soluble sugars. The addition of NaOH suspends and neutralizes the precipitated proteins. The product was then eventually frozen dried (Gupta and Nayak 2014).

Physicochemical Properties Soy Protein

The chemical ratio of soya protein is that it has both polar and nonpolar side chain. As a result, intra- and intermolecular interaction will be strengthened due to hydrogen bond formation, dipole interaction, and hydrophobic interactions. The stiffness, yield point, and limit strength of soy protein films are increased as a result of the side chains of soy protein molecules' strong charges and polar interactions with one another (Zhang and Jane 2001). In aqueous solution, pH 4.5 causes the precipitation of the soy protein, and hence it is decided as the isoelectric point for soy protein. Film formation at or near the isoelectric point due to coagulation was not reported. But it can be bypassed and deployed at pH values far from its quiescent point.

Therefore, their functional groups have been exposed and intermolecular interactions will increase. Therefore, it is clear that the mechanical properties of soil proteins can be affected by their pH. Physical properties during processing are better under alkaline conditions (Kim and Netravali 2011). Both soy protein and soy protein isolate were naturally brittle when processed without plasticizers or modifiers. The interaction between protein and some smaller molecules like plasticizers, water, lipids, and certain additives influences the mechanical strength of

network of proteins. The structural stability and mechanical behavior of SPI films are, on the other hand, influenced by various functions such as length of the polymer chains, water resistance, and charges in the surface of the polypeptide chains. SPI films have durable O₂-resistant properties and permeable parameters which are less than approximately 500 times in lower relative humidity atmospheres (Song et al. 2011).

Many methods are being investigated to enhance the functioning of soy protein, and one of them is to use natural fibers from a composite material to strengthen soy protein films. Due to the natural fibers' eco-friendliness, biodegradability, sustainability, low cost, and density, as well as their ability to be produced from waste streams, these composites with natural fiber incorporation are a desirable option. Utilizing additives like cross-linking agents and plasticizers to enhance the mechanical characteristics and moisture sensitivity of soy protein films is another method for combining protein with other natural polymers.

Methods to Improve the Mechanical Properties of Soy Protein

pH Treatment

Utsumi et al. (1997) reported that soy protein isolates have good gelating ability, and emulsification foaming and water absorption properties. It is poorly soluble in acidic pH condition. At isoelectric point, the SPI was almost insoluble. So at alkaline pH (8–11) that means far from the isoelectric point, the soy protein differs its film-forming capacity. At this pH, the denaturation of soy protein was promoted that leads to unfolding, and solubilization of protein assists the formation of rigid structures. The Kim and Netrasvali designed reinforced green composite with soy protein-hemp yarn. They also discovered that the interfacial shear strength of SPC resin/hemp yarn was raised with pH, rising from 17.7 MPa at pH 7 to 28.0 MPa at pH 10. As a result of the exposed reactive groups in the peptide chain caused by the addition of NaOH, the soy protein underwent another modification that boosted intermolecular contact with the hydroxyl group of the cellulose hemp yarn. If the alkaline state is exceeded past pH 10, soy protein molecules and electrostatic repulsive forces appear to grow, which reduces the interaction with cellulose fibers.

Thermal Treatment

Ryan et al. in 2008 studied that pH, protein concentration, and calcium supplementation affect the thermal stability of soy protein isolate and whey protein hydroxate. Thermal treatment is the oldest method used for the modification of soy protein. Thermal treatment decreases the action of protease inhibitors, eliminates lipoxygenase and volatile chemicals, and improves the functional qualities of the product (Barac et al. 2004). Studied thermal properties of soy protein isolates are investigated by thermo gravimetric (TG) and derivative TG (DTG) analysis.

Actually, SPI materials without any secondary components have no satisfactory physicochemical and mechanical properties for industrial-level applications (Park and Hettiarachchy 2000). Especially the soy protein biopolymers' thermal stability can be increased by blending with other 2 biodegradable polymers; chemicals or enzymatic modification has been applied (Pathak et al. 2009).

Yulong et al. have studied about the thermal degradation of sodium alginate-incorporated soya protein isolate/glycerol composite membranes. This was done by thermal gravimetric analysis method (TGA) and differential thermal analysis (DTA). The samples were heated from ambient up to 1050 K with temperature programmed at certain heating rates in air at a flow rate of 30 ml/min. Thermal degradation in oxidative atmosphere, the normal SPI- and SPI-SA-integrated biomaterials, exhibited a 3-stage decomposition. At 400 K, an initial weight loss occurred mainly due to the physical water loss from the sample, but during second-stage main weight loss happened. The pure SPI losses into weight in the temperature range from 540 to 630 K. But in SPI /SA composite, the main weight loss happened in the range of 480-600 K. Several reviews reported that the inter and intra molecular linkage in proteins is caused by treating the soy protein using heat at basic pH. Studies also revealed that the heating of SP films at 80–95 °C for various periods increased tensile strengths. The soy protein isolate seems to get aggregated via strong forces of interactions like hydrogen bond formation and disulfide bridges within the protein molecules and was experimentally proved by checking the solubilization of film heated at 65 °C for 6, 18, and 24 h and also at 50 °C for 6 h. It was also reported that the solubility founded more than 95% dissolution of the protein sample in the urea buffer. Here, the component urea serves as an H-bond breaker and 2 mercapto ethanol as an S-S bond-breaking agent. The resultant SDS-PAGE patterns of the respective proteins showed protein aggregates.

Tensile Strength Modification

Tensile tests are the most often used methods for figuring out the mechanical characteristics of biopolymer components as well as their structural layout. The ratio of stress to strain (stiffeners) in a material at the elastic stage of a tensile strength is known as the “Young's modulus” (Azammi et al. 2020). Due to the rigid, brittle physical properties and little flow ability soy protein isolate is reinforced with reducing agent, a starch filler, plasticizer, water etc. to increase the tensile properties. Studies revealed that soya protein concentrate reinforced with ramie fiber showed tensile properties similar to steel (Netravali and Chabba 2003). Soy protein films made from purely soy protein isolate bend to the brittle. So there is a need of converting them into composite from using functional nanomolecules. The dimension of nanoparticle approaches the Fermi wavelength of elusion and metal nanoclusters (NCs) where size is less than 2 nm as a novel functional nanomaterial. They are composed of a few to 100 atoms. Studies by

Husseinsyah et al. (2014) clearly studied that the tensile strength of SPI has increased by modifying with kapok husk films (KH).

The Kapok Husk filler, which serves as a reinforcing component, is to blame for the increase. From the SPI measurements to the Kapok Husk filler phase, it can facilitate the transfer of stress. This is a result of the hydroxyl and carbonyl groups in the SPI matrix and the hydroxyl group in the Kapok Husk filler forming intermolecular hydrogen bonds with one another. Huang and Netravali's experimental study from 2009 revealed that modified SPI with Kapok Husk filler and Glutaraldehyde reinforcement had greater tensile strength than unmodified SPI cross-link using reactions involving a Schiff base. This linkage strengthened the interaction on interfaces and the wetting property of SPI matrix and KH filler. The tensile strength of the glutaraldehyde-treated SPI films is better than that of untreated SPI films because of the imine linkages (Jin et al. 2013).

Water Barrier Properties Modification

The low-moisture barrier properties of the soy protein films arise due to their hydrophilic nature. Studies have been focused on minimizing the hydrophilicity and stabilizing the mechanical properties in order to enhance the physical characteristics of biopolymer bond films. Addition of hydrophobic chemicals is a new technique for enhancing biopolymer's moisture barrier qualities (Hernandez 1994). The moisture barrier properties of biopolymer films have been enhanced by the incorporation of lipid components like neutral lipids, fatty acids, or waxes. Studies also proved that additives of Eugenol (Eu) into SPI film improve the barrier properties. An alternative method to make soy protein films more functional is to alter the network arrangement of proteins majorly via cross-linking a protein chain. The soya protein isolate has low-oxygen permeability values, and this property provides an opportunity for preserving foods from oxidative deterioration. Experimental works also become successful by using glycyrrhizin alginate nanogel particles (GI-ALG NGPs) incorporated with SPI matrix which form a continuous and cohesive network and also act as a water barrier. In earlier times, the studies on rheology and water absorption properties of soy protein isolate with alginate have conducted many, but the nanogel particles in place of alginate is a recent work (Aritrasinha 2021), and it proved best as a moisture barrier.

Water-Holding Capacity Modification (WHC)

The term "WHC," which stands for "water holding capacity," refers to the total amount of water that can be absorbed per gram of protein powder. Actually, this is the result of proteins directly interacting with solutes like water. There are three distinct phases of water absorption that define soy protein's water absorption. The ionic and high-energy site surfaces on the polypeptide are bound by approximately

1 g of water for every 100 g of solid during the first phase, resulting in a highly structured monolayer. Apart from this monolayer, 25 g of water is hydrogen bound to the polar groups, changing the conformation and causing the protein matrix to swell at first. In essence, the polar group is connected to the water retention of proteins (Nguyen et al. 2015).

Blending

The best method to increase the physical properties of SPI materials, a practice of binding soy protein with polymers like polysaccharide proteins and synthetic, is followed which results in better products. Natural polymers used for blending are sodium alginate, calcium, cellulose, chitin, chitosan, glutan, whey protein isolate, and gelatin. Poly vinyl alcohol (PVA) is proved as a good blend for getting SPI film strength, flexible and better adhesive properties (Chen et al. 2013). Natural polymers are hydrophilic in nature, so the blends made by natural polymers cannot solve the moisture sensitivity problems of soy protein films. So the solution for this is to use synthetic polymers in place of natural polymers. Synthetic polymers like polyvinyl alcohol, poly lactic acid, and poly butylene adipate- co- tetra phthalate were used for blending and are biodegradable. Also they are hydrophobic in nature and do not have good interfaced adhesive (Chen and Zhang 2012). Another study using bioabsorbable polyphosphate filler as a blending agent revealed that this leads to changing the failed micromechanism of the blended soy plastic to a pseudo ductile way from being fragile. It enhances properties such as water resistance, strength, stiffness, etc., (Otaigbe and Adams 1997). Interpretation of gelatin also processes between successful blending agents. It results in gearing up the limited strength of elastic modulus and the increases the limit strength elastic modulus and ability to swell by the soy protein films. As a result, the transparency of the soy protein films increases and becomes easy to manage. Flexibility also increased. Some natural oils were also used as blending agents like Epoxydized soya bean oil (ESO), virgin extra olive oil, and lactic acid (LA) which were added to SPI-based films, and this improved hydrophobic character of film.

Plasticizers

The brittleness nature of SPI has been removed by adding plasticizers because they have the ability to reduce internal hydrogen bonding. Three theories are there to elucidate the effect of plasticizer mechanism: (a) Theory of Lubricity which says that plasticizers can enhance the interactive motions of macromolecules as does a lubricant; (b) Gel Theory which says that weak interactions such as H bonding, Vander Waals, and ionic bonds can be interrupted by polymeric interactions; (c) Free Volume Theory which says that an increase in the free volume of polymer can lead to depression of transition temperatures of glass. One of the basic perceptions underneath these theories is that behind these theories plasticizers are able to interpolate themselves between polymeric chains and can result in decreasing force of attraction which held the chains. The molecular weight and number and position of hydroxyl

groups of plasticizers are all variable that affects the ability to plasticize soybean protein-based polymer.

Wan et al. in 1996 proved that polyols from the ethylene glycol series are a good plasticizer due to their similar polarity to soy protein. Studies also proved different outcomes of plasticizers on the SPI film characteristics. Glycerol and sorbitol in 1:1 concentration result in the increase of moisture sorption content. Glycerol is found to be a good plasticizer, but adding 2,2-diphenol-2-hydroxy ethanoic acid (DPHEAC) and various amounts of salicylic acid again improves the properties of SPI like the increase in tensile strength and modulus with lower water sorption.

Surfactants

The most common surfactant used is Sodium Dodecyl Sulfate (SDS). A study of Sessa et al. proposed the effect of control of viscosity of zein by sodium dodecyl sulfate (SDS) during the time of thermal processing. In their experiment, they blend zein between water and TEG (Tri ethylene Glycol) followed by a heat treatment and shearing inside a torque rheometer to observe the change in viscosity. However, following the addition of SDS, there are reports explaining a diminishing tendency to the increase in viscosity of the zein/water/TEG in parallel to the addition of SDS. At the same time, when there is an increase in the quantity of SDS, the tensile strength and Young man modulus were found to be decreased with an elongation in bioplastics made up of zein. The application of SDS with soy protein isolate ensured a ductile material. Another study by Veerbeek and Van Den Berg in 2011 used a combination of SDS and urea for the processing of thermoplastic blood meal bioplastic extrusion. Results showed that the consolidation of the bioplastic was strongly dependent on the amount of SDS, and it is required for bioprocessing and consolidation. But in higher concentration, it hinders the formation of new intermolecular forces. Use of SDS leads to the improvement as process ability and the processing window get expanded. The blood meal is processed by injection molding with the addition of SDS and urea. Apart from SDS, adding different types of fatty acids like palmitic acid, stearic acid, and oleic acid in the film-forming solution is an efficient method to get thick SPI films with a white color and lesser chances of shrinking followed by drying of the film.

Biodegradable Polymers

Biodegradable polymers are low-cost good processability materials belonging to a family of polymer materials. There are 2 types of biodegradable polymers. They are natural and synthetic polymers. Natural polymers are basically polysaccharide or protein.

Chitin is a natural polymer having hard and elastic property which is employed for combining SPI in the presence of glycerol. Chitin has some rigidity which enhances the tensile strength and Young's modulus of SPI films (Denavi et al. 2009).

Chitosan is also a natural polymer made up of β -1,4 linkages between 2 amino 2 deoxy – D glucopyranose as monomers. It is used as a blending agent. It is less hydrophilic than SPI film. So its blending results an increase in water content angle and decrease in surface energy.

Propyleneglycolalginate (PGA) is a biodegradable polymer used to combine with SPI. The water stability of the film is improved by the formation of covalent bonds between propyleneglycolalginate and SPI. Total 10% addition of PGA is found to increase the resistance of water vapors and ductability of the film.

Cellulose is also a natural polymer which on adding SPI increases the oxygen and carbon dioxide barrier properties and also its mechanical strength. Apart from other blending agents, the cellulose needs a cosolvent due to its amorphous nature, so here 1-butyl-3-methylimidazolium chloride (BmimCl) is used.

Gelatin is a natural biodegradable polymer which on combination with soya proteins leads to a better blended product. The presence of sugar rings in the polysaccharide chain limits the flow and sliding moment of polymeric chain over each other. That increases the stiffness and brittleness. The porous nature of this combined form extend its application in the medical field very successfully in skin tissue regeneration purpose.

Caesin-incorporated SPI is also a better option to contribute to the features of SPI film. The transparency of the film can be increased by either sodium or calcium casienate. In addition, calcium caseinate also plays an important role in improving the elasticity and tensile strength of the blended films.

Poly lactic acid (PLA) is thermoplastic polyester used as a blending agent. SPI-PLA blends can be created using a screw extruder vent and poly (2-ethyl 2-oxazoline) as a compatibilizer.

Poly butylene succinate (PBS) is semicrystalline thermoplastic polyester with good elongation and physical properties. Direct blending of PSB with SPI is not so successful. So first, PBS is reacted with toluene diisocyanate (TDI) at different ratios to prepare methane group containing PBS. The remaining -NCO group on the PBS reacts further with the functional group in SPI when it is introduced.

Polycaprolactone (PCL) is a type of synthetic aliphatic polyester. The blending of PCL with SPI can be enhanced using some molecules having reactive ability such as MDI (Methylene Di pheny Di isocyanate) and TDI (Toluene 2, di isocyanate). Coconut oil also has plasticizing ability and compatibility in SPI and PCL blend and possesses highly effective mechanical properties, biodegradable properties, and adhesive properties.

Polyurethane (PU) is a blending agent having dual properties like elastomeric and thermoplastic character. An experimental work was done by Zhang et al. in 2003 by preparing prepolymers. A series of SPI-PU composites were created by compression molding a series of polyurethane prepolymers (PUP) that include NCO groups. The prepolymers' -NCO groups reacted with the SPI's -NO₂, -NH, and -OH groups, enhancing the blends' toughness, thermal stability, and water resistance. Additionally, ionic waterborne polyurethane (WPU) can be blended with SPI to enhance thermal stability, optical transmittance, and water resistance. Waterborne polyurethane, which is nontoxic and inflammable, increasingly replaced organic solvent-borne polyurethane once the environmental and health issues were taken into account.

Polyvinyl alcohol artificial polymer is hydrophilic. It is widely produced all throughout the world. In order to create biodegradable composites, it is mixed with organic polymers.

Effect of Plasticizers

Plasticizer is an addition that is added to a plastic or an elastomer to make the material softer or pliable. They actually break the intermolecular linking and thereby stabilizer protein in their primitive structure (Gurgel et al. 2011) and made the protein chains movable. Various researches were conducted for studying the effect of plasticizer. Due to its small size and hydrophilic character, glycerol is one of the most commonly utilized plasticizers for soy protein modification and has become increasingly compatible with soy protein. The influence of glycerol content on SPI plastic is investigated using Fourier Transform Infrared Analysis (FTIR), thermo gravimetric analysis (TGA), and differential scanning calorimetry (DSC). The results show that flexible films with good mechanical properties can contain between 25 and 35% of glycerol by weight. The tensile strength decreases as more glycerol is added, yet the elongation at break increases. This is as a result of glycerol's ability to reduce links between protein chains while enhancing chain mobility (Guerrero et al. 2014). A study was conducted using water as plasticizer for biopolymeric materials and was found to be effective (Reddy and Yang 2011). Plasticizers like stearic acid, sodium dodecyl sulfate, ferulic acid, waterborne polythene, etc. were reported to improve the water vapor barrier properties of soy protein.

Effect of Cross-Linking Agent

The mechanical and water resistance qualities of soy protein polymers are improved by yet another addition. Zinc sulfate, formaldehyde, acetic anhydride, glyoxal, and glutaraldehyde are a few examples of cross-linking agents. Studies showed that using glutaraldehyde (GA) as a cross-linking agent increased SPC strength and decreased moisture absorption (Chabba et al. 2005). In recent years, an unique natural cross-linking substance known as 1, 2, 3-propanetriol-diglycidyl-ether (PTGE), which is formaldehyde-free, practical, and easily accessible, has been added and shown to have an extremely high tensile strength.

Enzymatic Treatment

Enzymatic treatment for processing of soya protein is found to be a good method, for mainly oxidoreductase enzyme was used. The enzymes such as laccase (EC1.10.3.2), catechol oxidase (EC1.10.3.1), O-amino phenol oxidase (EC I. 10. 34) or peroxidase, glucose oxides (EC 1.1.3.4), hex oxidase (EC I. I. 3.5), or a cellobiose oxidase (EC1.1.3.25) were used for treatment. An oxidoreductase may be from plant, animal, bacteria, or fungi (including filamentous fungi and yeast). Some of the fungus-secreting laccase enzymes are *Aspergillus*, *Neurospora*, *T. Verscefan*, *M. thermophore*, *C. comaties*, *R. solani*, etc. Example for bacteria-secreting laccase is from the strain of *Bacillus*. Sometimes, oxido-reduction is produced by a method of cultivating a host cell shear forced with a recombinant DNA vector which carrier is a

DNA sequence encoding the oxidoreductase and also DNA sequence-encoding functions permitting the expression of the DNA sequence encoding the oxidoreductase, in a culture medium under conditions permitting the expression of the oxidoreductases. Studies using polyethylene glycol diacrylate (PEGDA) found a good concentration reduces the viscosity of soy protein and increases the water resistance capacity.

Incorporation of Other Biopolymers

Different types of cellulose can be incorporated with soy protein plastic to improve its mechanical properties. The type of biopolymers used for this purpose is commonly known as fillers. Microcrystalline, short fiber, and long fiber celluloses were the principal fillers utilized. The elongation, young's modulus, and tensile strength for soy protein without cellulose filler were reported to be 4.1%, 1481 MPa, and 37 MPa, respectively. In reality, adding microcrystalline cellulose to soy isolate promotes stiffness rather than tensile strength. Young's modulus rises along increasing short filler cellulose concentration, reaching a maximum of 2250 MPa at 20% cellulose. With 5% short fiber cellulose, elongation falls to 29%, and it slightly rises with 16 and 20% short fiber cellulose. When long fiber cellulose is added, the plastic becomes stiffer, has lower tensile strength, and has less elongation. Water absorption studies on these fibers' ability to absorb water also showed that soy proteins without fibers can absorb up to 32% of water after 26 h of immersion in water at ambient temperature. Soy protein with a 20% microcrystalline content absorbs about 28% of the water. Short-fiber cellulose increases water absorption to 34% when added at a 20% concentration, but only up to a 15% reduction in water absorption. So this rise may attribute to the fluffiness of the structure of the material that is because of the short fiber cellulose at this particular concentration. At the same time, no significant change in water absorption is noted in long fiber cellulose-incorporated soy protein.

Soy Protein Bio-nanocomposite

Soy protein isolate (SPI) gained significant attraction in the package technology field because of super easy biodegradability and film forming properties. But SPI-based films are always susceptible to inner mechanical functioning and higher sensitivity to moisture and limit its practical applications. Concerning these matters, several studies have been conducted to develop biobased nanocomposite films. Reinforcement units cellulose nanofiber (CNF) and nanosilica (NS) particles were studied, and results showed an increase in mechanical properties, aqueous services, barrier peptides, etc. of SPI-based composites. The CNF and NS particles were incorporated with soy protein film with low help of a silane reagent (KH 560). It bridges CNF and NS and forms a nanohybrid skeleton. It also enhances the interfacial interactions between nanofiller and SPI matrix by

exposing the exsposed functional group. This soy protein isolates cellulose nanofiller, and nanosilica nanocomposite films were developed. An experimental study was conducted using a copper nanocrystal for developing soy protein bio-nanocomposite. To improve the mechanical properties of SPI-based films, water-disposed CuNCs (Copper nanoclusters) were applied with the soy protein isolate with facile method. To improve the mechanical properties of SPI-based films, water-disposed CuNCs (Copper nano556 clusters) were applied with the soy protein isolate with facile method and along with chitosan is also attached. When checking about the tensile strength and EB (Elongation break) values of SPI -CS - CuNCs films, they were about 118.78% and 74.93% increased, respectively. The compatibility between SPI and chitosan was found to be increased by adding CuNC, hence micro structure of the SPI-based film becomes more homogeneous and the hydrophobicity and thermal stability also get better. The water vapor barrier ability got decreased, and so the nanocomposite film is found best in food-packaging materials. Nanocomposite films were formulated isolates of soy, clays (Na + -MMT), and eugenol (antimicrobial agent). The shear thinning behavior and consistency in SPI enhance with the addition of Na + MMT. Because it is inexpensive and readily available, nanocomposite (MMT) is a popular nanoparticle for soy protein plastic. Melt extrusion of SPI/Montmorillonite (MMT) bio-nanocomposite films revealed appreciable improvements in the films' tensile strength, storage modulus, water barrier performance, and thermal stability. The pH of film-forming solutions essentially determines which qualities are superior criteria for extrusion and MMT content (Kumar et al. 2010). The soy protein isolate/cellulose sans composite's tensile strength and young's modules were improved as a consequence of an experiment using cellulose whiskers to strengthen the soy protein isolate. Since the cellulose whisker content is increased, the thermal stability and water resistance capacity of the SOPI matrix increased, and the reason behind this phenomenon was found to be the intermolecular hydrogen-bonding interaction between the cellulose whiskers and the SPI matrix (Wang et al. 2006).

Soy Plastics

Polyurethane and polyester thermoset includes the common types of soy bean-derived plastics. Soy polyols, which are polymers found in soybean oil, are used to manufacture adhesives, coatings, sealants, inks, and car panels, among other things. Polyols stability and endurance can be improved by using the right ingredients in their formulation. Due to the inclusion of various compounds that lend durability and strength to the final products, soy-based plastics appear to be nonbiodegradable in some situations. Soy protein plastics are made at considerably lower temperatures than conventional plastics, it appear and feel like petroleum-based plastics. They are generally are freezer safe and may tolerate hot foods and liquids up to around 90 degree celsius. Polymerization is a chemical process that involves the discovery of monomers in the presence of heat or a change in pH,

resulting in huge networks of linked chains known as polymers. The same thing happens with bioplastics, but they must first be treated before they can be used. In the case of soya, a variety of derivatives can be employed to make plastic. 60 pounds of soy generates 11 pounds of oil, 48 pounds of soy meal and/or 38 pounds of fat-removed soy flour, 20 pounds of protein concentrate, and 12 pounds of soy protein isolate. After dehulling and meal grinding, dehulled soybeans are ground into soy flour, which is subsequently loaded with varying degrees of protein solubility. Soy protein concentrate is made from soy flour that has had the water- or alcohol-soluble carbohydrates leached out of it. The next step is acidification of soy protein concentrate to pH 4.5 followed by dissolving the soy protein concentrate in caustic 12 solution of pH 9. That forms the purest soy protein in the market containing 90% protein and virtually ready to be converted into a plastic, but there is one additional thing to think about: the moisture content. Ford's team (who invented the world's first plastic automobile) found that if plastic swells are left in the rain, moisture content affects the mechanical properties of the final material. An option for controlling moisture content is by adding plasticizers such as glycerol or by oven or air drying methods. Once the moisture content is controlled, then the protein isolate can be tuned into a plastic using heat and pressure. At the same time, compression modeling and extrusion are also done. Many chemical modifications have been made during processing to improve the mechanical properties and moisture sensitivity of soy protein plastics (Nanda et al. [2022](#)).

Methods for the Formulation of Soy Protein Product

Soluble sugars are typically left out of defatted soya flakes, or flocs, when making soy protein concentrates. The majority of the remaining substance is made up of insoluble polysaccharides and proteins. The remaining soy product's protein level increased naturally as a result of the removal of soluble sugars, and the unwanted oligosaccharides that cause flatulence were also removed. To remove soluble sugars and produce soy protein concentrates, a variety of leaching techniques are being investigated. The starting material's quality, the yield, and the extraction conditions all have an impact on the leaching process. Hexane was used to extract the oil from the whole flat flakes of soy to create soya concentrate. For improving the quality of the product, research study by Russin et al. in [2011](#) reported that a solvent mixture of hexane, ethanol, and water can be used for the oil extraction of full flat flakes. It has also had the capacity to remove substantial amount of undesirable glycerides, phosphatides, and off-flavor components which are not removed during hexane extraction. Heat is an abiotic factor which affects the quality and yield of soya protein concentrate. Excessive toasting of defatted flakes leads to reduce the amount of soluble sugars to leach out, and that results in lesser amount of protein content in the final product. There are three methods for manufacturing soya protein concentrate aqueous wash, acid wash, hot water leaching, and enzymatic treatment:

Aqueous Alcohol Wash

This method is employed in case of commercial production of soy protein concentrate. Total 50 to 70% of aqueous alcohol is used in this method, and it extracts soluble sugar and small amount of soluble protein. As the aqueous alcohol has the capacity to denature the protein, the protein becomes insoluble and the insoluble polysaccharides also remain. The alcohol is finally recovered by flash desolventizing. The water/alcohol ratio is an interesting parameter for controlling the protein composition, yield, and functional properties of the SPC. The most interesting functional properties were obtained when using either water-only solvent or solvent with high water/alcohol ratios.

Hot Water Leaching

Heat denatures proteins, and soy protein does too, rendering it insoluble in water. Moist heat has greater denaturation potential than dry heat. Small molecular weight components, such as soluble sugars, were removed from the insoluble protein and polysaccharide matrix using high temperature-heated water. The quality of soy protein products is crucial. This trait can be found in things like the ability to influence flavor, texture, and water binding.

Application of Soy Protein

In Biomedical Field

The lower price, vegan nature, guaranteed safety from transmissible diseases, longer shelf life, and permanence, and biodegradable abilities gain the attraction of soy protein as a cheaper and best source of biopolymers (Deepmala et al. 2017). The applications of biopolymers in medical field include revitalization of tissues, preparation of biologically active bone fillers, and dressings for mini and major surgeries. It has the capacity to incorporate them into the blood clot and enhances the responses of collagens leading to countable depositions with great potential for healing of wounds. Soya hydrogel manufactured by various processes is getting popularized for delivery of molecules and other clinical needs. In the studies of Synders et al., combination of soy protein and PEG (Poly ethylene glycol) hydrogel can be used to prepare wet mout wound dressing and also can be employed for transdermal drug delivery system. The ability to incorporate into various drugs by soy protein biopolymers is greatly enhanced by augmenting it with polar and nonpolar amino acids in a modest ratio (Chien et al. 2014).

For Wound Healing

A wound is an injury that causes an external or internal rupture of body tissue, usually skin. Based on the type of repair mechanism, wounds are divided into acute (mechanical injuries and burns) and chronic wounds. Acute wounds generally heal within 8–12 weeks with minimal scarring. Because of the long duration for breakages, wound healing time is not found to be satisfiable for chronic wounds. The cascade of healing is classified into four overlapping phases like homeostasis, inflammation, proliferative, and maturation. The first response to injury is to build damaged blood vessels and activate platelets to form fibrin clots, which stop blood flow and create a scaffold for incoming inflammatory cells. Neutrophils are immediately recruited to the clot as the first line of defense against bacteria. Monocytes are recruited within 48–96 h after injury and converted to tissue-activated macrophages at the site of injury. Macrophages and fibroblasts release various growth factors to aid wound healing and generate granulation tissue. Healing time depends on the depth, size, type, location, and stage of infection of the wound. Apart from that, it also depends on the health condition and age of the patient. A potent wound dressing should manage the wound without causing a secondary infection and should be able to create an aseptic enA potent wound dressing should manage the wound without causing a secondary infection. It absorbs exudates from wound, resists wound desiccation, stimulates growth factors, stays elastic and flexible, and also is antigenic and biocompatible. Conventional wound dressings are designed to create a closed wound environment to prevent infection and a moist environment to facilitate the wound-healing process. Recent discoveries in the development of advanced wound dressings have demonstrated the use of materials or incorporation of biologics that can promote wound healing events ranging from cell migration to generation of extracellular matrix components. Another option for wound healing is by applying tissue grafts, but studies reported that it has some limitation because of rejection or possibility of transferring diseases, and also they are very expensive. Because of the characteristics nature like high biodegradability, biocompatibility, and similar property of extra cellular matrix components the natural polymers have high demand in use as wound dressing materials. Among these plant-derived proteins, soy has been considered for burns and wound dressings. It was studied that reepithelialization at the wound site is affected by the inflammatory response of the wound tissue because of the dressing materials, so it can resist inflammatory responses at the early stage, and so scar formation possibility can be reduced. In this particular context, the genistein and daidzein commonly known as phytoestrogen is well known in promoting the wound-healing attributes during the inflammation mechanism mainly due to the immune modulatory activities (Pierzynowska et al. 2021). By this way, the activity of lymphocytes, macrophages, and monocytes can be reduced. It promotes the blood clotting and deposition of collagen to start the healing process. Different forms of soy proteins were studied as the application of soy protein in wound dressing. Some of them are discussed below:

(A) Films

Soy protein isolate films are very brittle and are made by solvent casting. The globular structure and internal hydrogen bonding between chain is especially the strong inter- and intrainteraction between components which is the reason for easy brittling. A better way to improve the flexibility is by adding plasticizers such as glycerol, sorbitol, etc. Biodegradable blending agents like sodium dodecyl sulfate and polycaprolactone- triol are also used. In vitro studies revealed that plasticizers will increase the flexibility and ductility of the films as they have the hydroxyl components and they can reduce the internal hydrogen bonding between the polymer chains and initiate the space area between the molecules (Suyatma et al. 2005). Blended with hydrophobic polymers leads to the effect on water absorption properties and degradation behavior. In addition to this, a combination of chitosan and soy protein membranes is also used in the medical field as wound dressings. Silva et al. studied on such blending and added glutaraldehyde to function as a cross-linking agent to get biologically compact membrane for wound dressings. In vivo studies in wounded rats model resulted in the combination of chitosan-soy protein which can induce fresh tissues to form which can accelerate the healing of wound when compared to control. They also reported that the chitosan soy protein combination improves induced anti-inflammatory properties and positive host responses which also trigger wound healing.

(B) Hydrogels

Conventional wound-dressing materials like bandage and gauzes are effective in controlling hemorrhages, but they have limitations that they are non-biodegradable and susceptible to infection. Another drawback is it is not suitable for shapeless wounds, it uses secondary tissue infections, and it is found ineffective for wound healing. We can overcome these limitations by using hydrogels which can manage moist-free wound conditions because of the water-absorbing nature of the materials. It also has a positive impact regarding the oxygen permeability, and thus it provides a barrier against bacteria. Studies by Synders et al. revealed that only soy powder mixed in water can be formed to gel form without adding any cross-linking agent. In drug delivery system, also these hydrogels were used nowadays. Total 12% of soy protein concentration is better for making hydrogels because in this higher concentration the network is improved and modified and thereby the mechanical properties were automatically improved. The residues from soy protein hydrogel degradation are non-toxic and were shown to excite deposition of collagens in the fibroblastic cells of culture. This proves that soy protein has intrinsic bioactivity. Commercially, the most popular hydrogel type is the alginate hydrogel (Tansaz et al. 2016).

(C) Fibers

Micro- or nanofibers are made from soy polymer by electrospinning technique. Electro-spun mats are best suited for wound healing because of their high surface area and porosity nature (Ratchada et al. 2017). Soy protein blending using PEO (poly ethylene oxide) in various concentrations such as 20:80; 40:60; and 60:40 helped in the fabrication of fibers. The efficiency of fibers were tested

by analyzing the moisture vapor transmission rate (MVTR). The basic mechanism behind the water absorption property of soy protein is that increased concentration of soy protein can decrease the MVTR. Thus it creates an atmosphere of moistness for healing of wounds. Antibacterial test is found to be most promising: The activity of SOY-PEO fibers inhibits the growth of *S. aureus* and *P. aeruginosa* (Phillips et al. 2015). Alginate SPI blend fibers were coagulated with calcium chloride; hydrochloric acid and ethanol were used in wound dressings. As the concentration of ethanol is increased, the mechanical properties of fibers in the wet state also increased.

In the Food-Packaging Field

Due to ready availability, unique structure property, and renewable character, soy protein is used in the food-packaging field. Bioplastics can be made from soy protein. So it is considered as a natural plastic that can form an amorphous 3D structure. Consumers always try to find compostable or biodegradable packaging material, so they can just dispose of the plastic or put it in green bins after use, and there is no need to worry about spending more energy in recycling the material. Biodegradable soy protein thus offers an option for consumers to choose “healthy packaging” thus creating an opportunity to reduce one’s carbon foot print and dependence on petroleum-based plastics.

Used As Adhesives

The adhesive property of soy protein helps to replace formaldehyde used in making wood-based panels used in house construction. Products made with UF resins like particle board, hard wood plywood, and paneling contain the chemical formaldehyde. Most of the consumer products contain formaldehyde-based resins that release formaldehyde and cause health-related problems. However soybean-based adhesives have been used to replace UF to reduce the emission of formaldehyde (Jisoo Jeon et al. 2011). Soy protein-Kymene[®], Soy baby[®] and Ozero[®], Soyad[™], and Cargill’s Prolia[™] are some commercially available soy-based adhesives. The volatile organic compounds present in the products will be reduced by the activity of soy ingredients in the adhesives used. It is also important to know that adhesives made by soy protein possess significant resistance to water with a wide range of binding capacity. The main advantage of soya adhesives was as follows:

- A soy-derived bonding adhesive designed to replace formaldehyde-based adhesives in fiberglass insulation applications.
- A combination of low cost soy phenol formaldehyde resins for core-oriented particle board applications. Up to 20% replacement of powdered PF and p-MDI by soybean flour in OSB boards was achieved without significant loss of overall properties, including moisture property performance.

- Improvement of soybean properties in wood bonding by jet cooking.
- Soy flour replaces animal blood-derived protein as blowing agent in phenol-formaldehyde resin, foam adhesive extrusion system for laminated plywood veneers.
- Formaldehyde-free resins containing soy flour or meal in wood composite systems such as plywood, panels, plywood, and parquet.
- A chemically modified soy protein-based adhesive that creates a strong, heat-resistant bond when bonding paper substrates.
- Liquid fast-curing soy-based adhesives for packaging.
- A strong, sound absorbing, easy-to-lay soy-based floor adhesive.

Conclusion

Soy protein is proved to be one of the best plant proteins used as biopolymer. Its flexibility in changing the chemical structure using various chemical agents makes it more strong and durable. Soy products can be made from soya using physical, chemical or biological, or enzymatic method. Making soy plastic and further modifying it using chemicals is also a marked characteristic feature. By modifying the soy protein polymer product to some extent, we can increase its mechanical strength that makes it a powerful ecofriendly material. The emerging fields of applications of soy protein in biomedical and material industry in the last few years indicate that research efforts in this area will increase in the near future application of soy-protein-based products. The recent development of soy protein applications in the biomedical and food materials industries suggests that future soy-protein-based products will see a rise in research efforts in this arena.

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Part III

Biopolymers: Properties

Piezoelectric Properties of Biopolymers

8

R. Divya, Swathi Yempally, and Deepalekshmi Ponnamma

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Abstract

Biopolymers with piezoelectric properties are widely attractive nowadays owing to their direct and indirect effects of piezoelectric behavior in addition to biocompatibility and biodegradability. Although a wide range of polymers such as polyvinylidene fluoride, are used to enhance the power generation, conversion efficiency, and storage capacity of different piezoelectric devices, biocompatibility is the significant property when bio-nanogenerators are considered. Biopolymers are notable for their wide availability, ease of handling and biodegradability, however durability and poor mechanical strength are some of the challenges associated with them. This chapter briefly illustrates the recent advances in developing biopolymer nanocomposites for piezoelectric applications and the influence of different nanomaterials in regulating the piezoelectric properties of biopolymers.

R. Divya

Department of Electronics and Communication Engineering, Sree Narayana Institute of Technology, Adoor, Affiliated to A P J Abdul Kalam Technological University Kerala, Thiruvananthapuram, India

S. Yempally

Center for Advanced Materials, Qatar University, Doha, Qatar

D. Ponnamma (✉)

Materials Science and Technology Program, Department of Mathematics, Statistics and Physics, College of Arts and Sciences, Qatar University, Doha, Qatar
e-mail: deepalekshmi@qu.edu.qa

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Biopolymer nanocomposites · Piezoelectric · Cellulose · PVDF

Introduction

With new technological advancements in day-to-day life, the strength, resources, and environmental issues faced by people are becoming increasingly extreme. Owing to the resource limitations of conventional nonrenewable power sources and the environmental damage they cause when in use, renewable energy is a popular area for development and research. Accordingly, different electricity assets together with solar energy, wind power, and ocean strength have been advanced hastily, but they nonetheless have positive boundaries inside the method of series and operation. In particular, they are unable to miniaturize or sensitize in any way.

The piezoelectric nanogenerator (PENG) is constructed with a piezoelectric material because it has evolved to collate and transform mechanical energy into electrical energy. Piezoelectric ceramics, such as lead zirconate titanate (PZT) (Hubbe et al. 2008; Mariano et al. 2014), barium titanium oxide (Hernández-Flores et al. 2020; Harrison et al. 2014), zinc oxide (ZnO) (Lu et al. 2019), and piezoelectric polymers, such as polyvinylidene fluoride (PVDF) (Lin et al. 2008; Xue et al. 2012), polyamide (PA) (Prokhorov et al. 2020; Qi et al. 2018), are the most common types of piezoelectric materials. The outstanding contribution of these compounds is the wide usage of piezoelectric mills in electricity harvesting, in biomedical domains such as high-risk heart diseases such as implantable, self-powered sensors and in wearable electronics. Wearable and implantable self-powered sensor devices require raw materials to be nontoxic, exceedingly flexible, biodegradable, and harmless to humans. Piezoelectric and triboelectric gadgets based on flexible natural polymers with the described advantages are advanced choices and involve a variety of considerations (Jahan et al. 2018). But, in addition, they also have certain risks, which include ecological issues, terrible biocompatibility, excessive cost, and nonrenewability, which limit their huge utility. Therefore, the improvement of excessive overall performance, renewability, good value, recyclable, and biocompatibility, and thus natural piezoelectric composite materials with biopolymers and PENGs, has been growing (Kopycinska et al. 2003). This chapter includes a few recent case studies on the piezoelectric properties of certain biopolymers such as cellulose, chitosan, and polylactic acid, etc.

Piezoelectricity at a Glance

The electricity that is the by-product of any electro-mechanical interactions is termed piezoelectricity and is basically the result of electrical and mechanical fluctuations. The materials that have no inversion symmetry for the mentioned parameters generate a transducer effect according to their need for mechanical stress. A material can produce a mechanical compression after it is subjected to an electric field, owing

to the centrosymmetric quality, and the converse piezoelectric behavior is always reasonable. Piezoelectricity can be scientifically denoted as a linear relationship between the mechanical factors of strain, compliance, and stress, and the electrical parameters of electric movement, permeability, and the intensity of the electric field. Generally, choosing of the exact crystal class and polymer to boost the mechanical requirements collectively yield implausible consequences in the design (Harrison et al. 2014). A substance is said to be piezoelectric if it can generate an electrical charge in response to mechanical stress. The term piezoelectric is extracted from a Greek word “*piezein*,” which relates the compression or pressure, and *piezo*, the Greek word for “push.” One of the distinguishing features of the piezoelectric effect is that it is conversable, that is the substances exhibiting direct piezoelectric effect, (production of electricity with the applied stress) shows the reverse piezoelectric behavior (formation of stress with the applied electric field).

Piezoelectricity, can also be defined as pressure electricity, by which the production of electric separation is spread over a mechanical stress. Ceramics, which commonly show high piezoelectric strain constant, and have many peculiarities such as hardness, elasticity, being lightweight, and piezoelectric stress constant, which is lower in natural polymers. Overall, a substance must possess stable molecular dipoles, have the ability to arrange or adapt the dipoles, be able to sustain the dipole orientation, and be able to withstand high stresses depending on the mechanical stress being applied (Ponnamma et al. 2017). The semicrystalline structured polymers have different advantages such as mechanical arrangement, polymorphic phases, thermal annealing, and excessive voltage treatment, bringing crystalline phase alteration close to the piezoelectricity outcomes.

The piezoelectric coefficient and piezoelectricity mechanism in a semicrystalline polymer are illustrated in Fig. 1. When a square crystalline material with either negative or positive charges is squeezed, there is no change in polarization. This is because the occurrence in the relative center of charges remains exactly at the same point. For a hexagon, given stress varies like the center of charge, thereby developing the variation in polarization. As the majority of semicrystalline structured polymers contain randomly organized tiny crystals in an amorphous framework, some crystallites are reoriented using poling techniques.

Figure 2 illustrates piezoelectric polymers such as bulk piezopolymers, which are classified as two. They are semicrystalline structured polymers and piezoelectric; next are amorphous dipolar polymers or polymer composites, which are certain piezoelectric particles implanted in any polymer or any piezoelectric pillars within a polymer and a voided charged polymer. In corona poling of the composite film, fibers from the same polar poly- γ -benzyl-L-glutamate (PBLG)/poly (methylmethacrylate) biopolymer composite film and piezoelectric films were reported (Cernea et al. 2012). Such composite provided 20 pC/N piezo coefficient d_{33} value. The fibers were oriented and were compared with the electrospinning process, then accomplished approximately 100% orientation of those electric dipoles inside the given structure of PBLG. Similarly piezoelectric characteristics of composite films are enhanced with different kinds of fiber reinforcement.

Fig. 1 The 2D crystal structures of the piezoelectric hexagon and nonpiezoelectric square displayed as examples of piezoelectric transduction modes

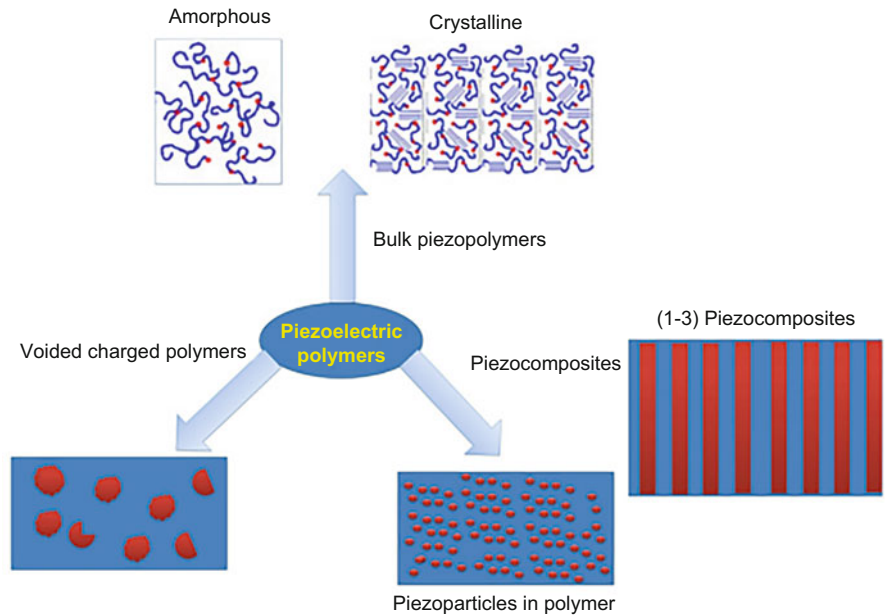
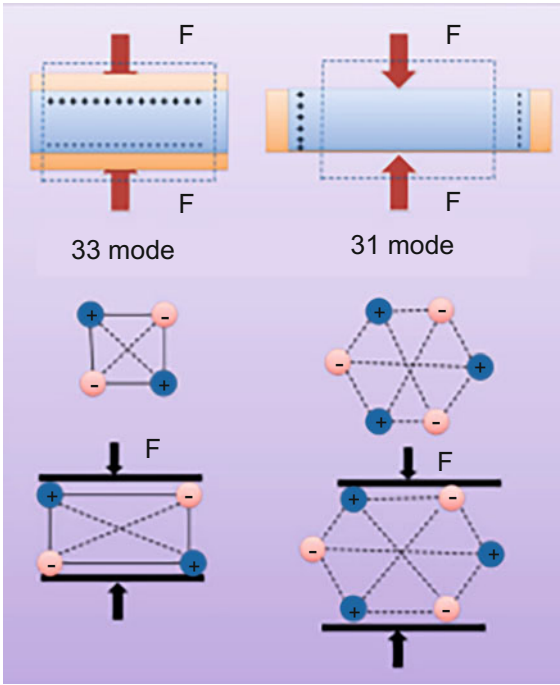


Fig. 2 The representation of various types of piezoelectric polymer

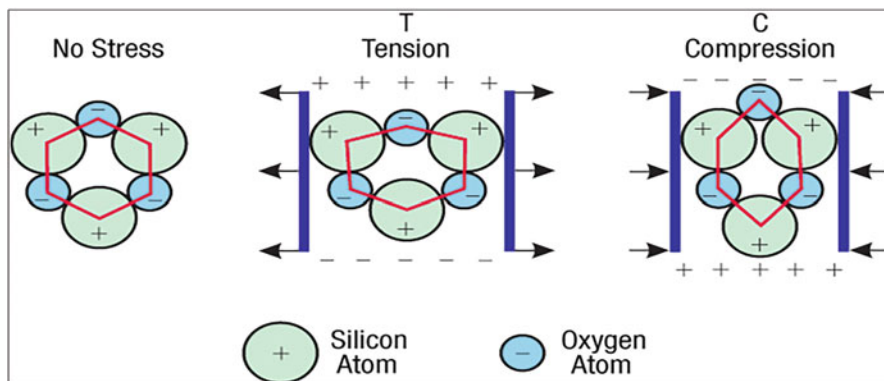


Fig. 3 The piezoelectric effect in quartz

The positive and negative charge centers within a piezoelectric material move in response to mechanical stress, creating an external electric field. The piezoelectric material is stretched or compressed when the direction of the external electric field is reversed. Piezoelectric effect is necessary in a variety of applications that include sound production and identification, high voltage production, extremely fine electronic frequency generation, and microbalances optical assembly observation. It also serves as the foundation for many atomic-resolution scientific instrumentation techniques, including scanning probe microscope (Fig. 3).

Piezoelectric effects are also used in more general applications, such as ignition sources in cigarette lighters. Free limits of volumetric expansion or contraction can preserve piezoelectricity and potentially improve nanometer-scale performance (Lu et al. 2019).

Piezoelectric Properties of Biopolymers and Nanocomposites

Nanocellulose has become a notable biomaterial in recent times because of the outstanding mechanical properties, renewability, biocompatibility, low cost, etc. (Prokhorov et al. 2020). Because of great abundance in nature, wood is an available base substance for the preparation of nanocellulose. However, the separation normally needs a process of a different stage that includes intensive chemical as well as mechanical processing for cellulose nanofibers, crystalline whiskers, and any other nearby structures of pure cellulose, those having a minimum dimension range of 1–100 nm (Hubbe et al. 2008). This makes the cellulosic material preparations very complicated and time consuming. The cellulose nanocomposites further show distinct qualities, because of the nanometric size effect in bulk media compared with conventional composites, even at low concentrations (Mariano et al. 2014). Properties and specialties of such nanocomposites include extreme chemical stability, mechanical properties, bio-compatibility, low cytotoxicity, cost effectiveness, and availability (Joseph et al. 2020).

It is simple to enhance the piezoelectric property of PVDF by using graphene (Xue et al. 2012). The solution casting method was used to create (rGO)/PVDF nanocomposites with rGO content ranging from 0.0 wt% to 2 wt%. A nanocomposite film was strained at a ratio of 4:5 and polarized using a step-field method to create a piezoelectric-phase crystal structure. The output voltages of the rGO/PVDF nanocomposite films were evaluated using substantial vibration tests and several experiments to examine the characteristics of developed piezoelectric. Compared with other fillers in the nanocomposite film, rGO at 0.05 wt%, possesses a significant voltage output that is roughly 293% of the pure PVDF. In addition, the output voltage enhances with increasing rGO content from 0 wt% to 0.2 wt%. More β -crystalline phase forms in these rGO depositions, which is confirmed by FTIR and powdered XRD spectrum analyses.

Recently emerging piezoelectric materials provide green solutions for the development of lightweight, biocompatible, flexible, and ecologically adaptable mechanical harvest energy. Such devices are used as independent power supplies for robotics, electronic skin sensors, and medical monitoring systems (Song et al. 2021). PENGs can use mechanical vibrations to power liquid crystal displays and LED by harvesting energy from human activity and sound waves (AlAhzm et al. 2021). The piezoelectric properties of composite fibers were tested the implementation of nanogenerators using PET substrates, aluminum foil, copper wires and similar components, and rectangular fibers (25×25 mm) were arranged parallel in between the copper wires and aluminum foils and then connected using a conducting tape on either parts. Usually, PET substrates were typically wrapped on the two sides of the given model using an adhesive on both sides. The PENG was tested for piezoelectric output voltage by testing under a load of 2.5 N and a resistance of 1 Mega Ohm. As the vibration frequency increases, the output voltage rises until it reaches a point where the voltage is balanced. In comparison with all other composite fibers, PENG manufactured from 3% composition of the hybrid nanofiller performs exceptionally well, which is due to the filler's ability to strengthen the material.

The excellent piezoelectric and ferroelectric properties of PVDF have led to practical applications in the fields of flexible detectors, sensors, and actuators in the up-to-date information and industries of electronics in recent years. The nonpolar phase is the most advanced stable crystalline phase, whereas the polar phase with the highest dipolar density and the strongest piezoelectric property.

The effect of the hybrid filler's micro- CaCO_3 content (Jahan et al. 2018) and subsequent uniaxial stretching at various ratios R (4–5) of elongation on the electrical, piezoelectric, mechanical, and dielectric characteristics of the hybrid nanocomposites. A hybrid nanocomposite typically consists of 40 parts CaCO_3 and 3 parts (wt.) of Mt., with a d_{33} of 7.4 pC/N, which is a high value for piezoelectric coefficient. The pressurized-type hybrid nanocomposites dependably exhibited an enhanced piezoelectricity with the greatest d_{33} value of 30.6 pC/N formed at R of 5, and it was assigned to the almost 100% β phase content and dipolar arrangement formed by extending and subsequent poling. Melt extrusion manufacturing for the hybrid filler added to PVDF nanocomposite has been illustrated with enhanced

piezoelectric properties and then it was extended to different draw ratios, R. Hybrid fillers always contribute to enhance the polar β phase in PVDF composites.

The metal oxides permit the electrical energy transfer and mechanical vibration transfer by forming interconnected networks throughout a particularly selected sample. This occurs as the ZnO and FeO filler materials have ferroelectric characteristics and distinct dipole orientation helping to produce the piezoelectricity. Electrospinning always permits the dipole alignment, and is responsible for the piezoelectricity. The dipoles in the ZnO and FeO subsidize to the improved piezoelectric performance. Furthermore, the nanoparticles act as stress junctions and differentiate the electrospun PVDF fibers into different segments. This increases the local deformation and makes the piezoelectric output voltage very large.

Cellulose nanofibers and nanocomposites are notable for exhibiting mechanical energy harvesting properties or piezoelectricity (Kalia et al. 2011). Composite material with an isolation structure has excellent electrical properties (Qi et al. 2018). At a frequency range of about 1 kHz, the polyamide/barium titanate/carbon nanotube ternary nanocomposites (PBCNCs)-3D components displayed a dielectric constant of 16.2, which is regarded as being two times greater than that of SLS-printed composite parts (PBCNCs) without an isolated structure. After comparing with PBCNCs, the strain coefficient of piezoelectricity increased from 1.7 to 2.1 pC/N. These outcomes will not only provide a way for the construction of 3D segregated percolation channels, which will improve the overall performance of polymer composites powered by piezoelectricity, in addition to offering a new material for SLS processing (Fig. 4).

Zinc oxide nanoparticles (ZnO-NPs) are one of the most eye-catching materials because of their exceptional optical, antibacterial, piezoelectric, and mechanical capabilities (Prokhorov et al. 2020). The dielectric, mechanical, and piezoelectric properties of chitosan nanocomposites can be varied by altering the concentration of ZnO-NPs in accordance with the applications. The structural characteristics of CS-ZnO films, including their conductivity, mechanical, dielectric and piezoelectric properties, can be changed by varying the concentration of ZnO-NPs. Moreover, CS-ZnO films with 15% NPs show a greater piezoelectric coefficient d_{33} value than films with 20% NPs (Fig. 5).

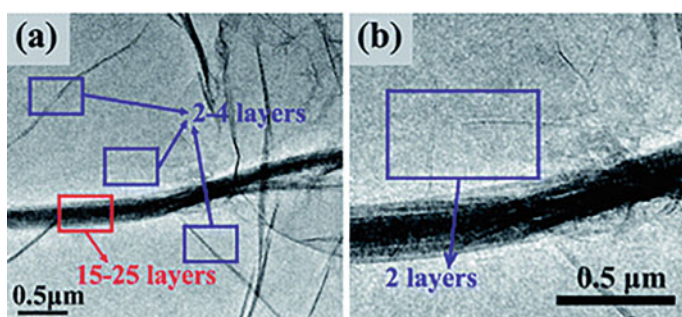


Fig. 4 RGO-PVDF nanocomposite films

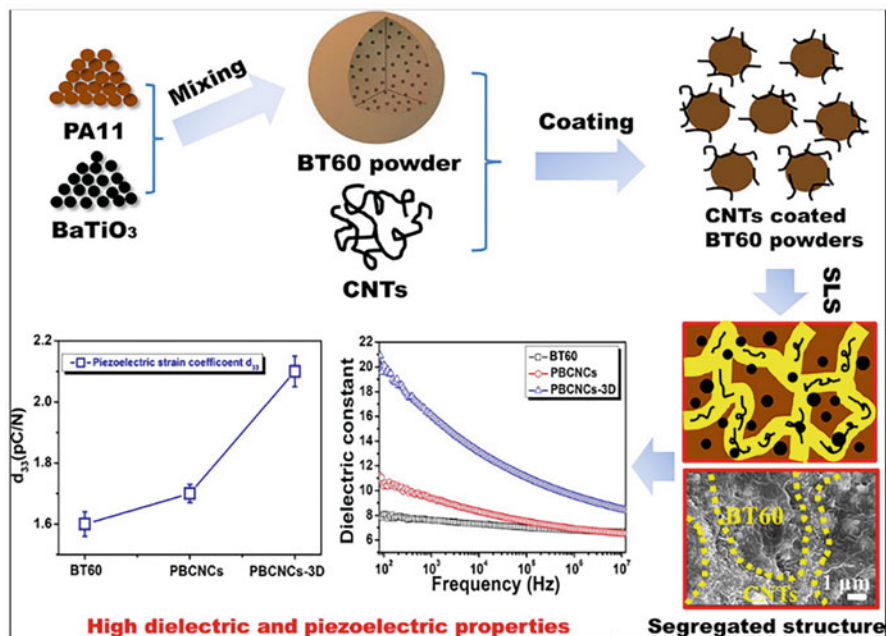


Fig. 5 Piezoelectric and dielectric characteristics in ternary polyamide11/BaTiO₃/CNT nanocomposites sintered by a selective laser

(Figure 6) shows the operation of a PENG based on boron nitride nano tubes (BNNT)-ZnO quantum dot (QD) (Shim et al. 2022) nanocomposites. Compared with BNNT, the features of BNNT-ZnO QDs' piezoelectric coefficient rise significantly. For various piezoelectric applications, the unique features of BNNTs can be demoralized. In contrast to the longitudinal direction, it is still exceedingly challenging to improve the piezoelectric capabilities in the radial direction. To extract the performance of piezoelectric characteristics, a design utilizing BNNT inorganic composites with outstanding inorganic piezoelectric materials is required.

The piezoelectric constants of the BNNT-ZnO QD nanocomposites and the distinction between out-of-plane and in-plane piezoelectric behavior were examined using traditional piezoelectric force microscopy (PFM) analysis in relation to the significant piezoelectric properties. Furthermore, using the SEM-PFM technique, the piezoelectric effect is induced by applying a specific radial force to a single wire of the BNNT-ZnO-QD nanocomposite. The effective single-line piezoelectric coefficient of BNNT-ZnO-QDs and the radial increases in the piezoelectric charge coefficient of BNNT-ZnO-QD films were 41.9% (-60.3 pC/N) compared with a single wire of BNNT and BNNT foil. At a low volume of 0.18 wt%, a transparent and flexible piezoelectric device based on BNNT-ZnO-QD nanocomposites exhibits excellent piezoelectric properties. The performance of PENGs using electrically poled BNNT-ZnO-QD nanocomposites improved by more than 140% and 45% compared to native BNNT-ZnO-QD nanocomposites and BNNTs respectively.

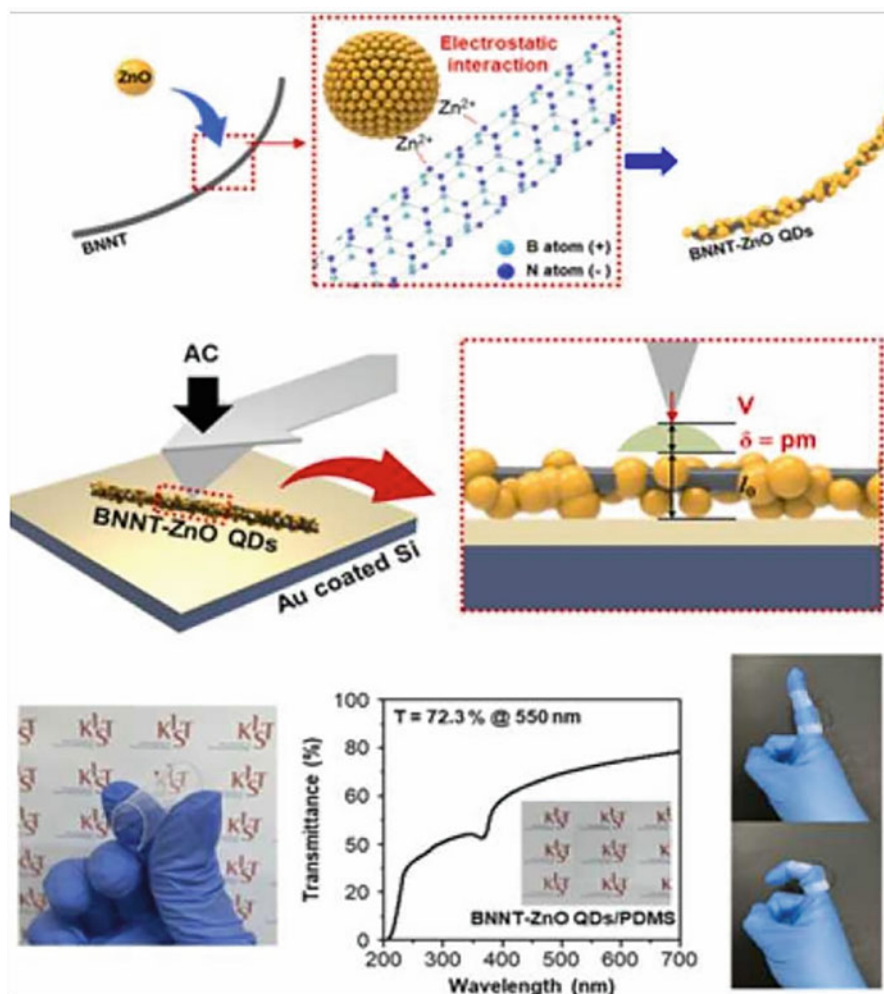


Fig. 6 BNNT-ZnO-QD nanocomposites for enhancing PENG and boron nitride nanotube piezoelectric properties

Therefore, there are new avenues for developing innovative materials and devices for generating renewable energy (Fig. 6).

Milled nanocomposite parts made of PA11/BaTiO₃ (Qi et al. 2017) possessed dielectric and piezoelectric properties. The solid-state shear milling technique improved the sintering window of the PA11/BaTiO₃ nanocomposites, especially for the nanocomposites containing 40% BaTiO₃ particles, and the broad sintering window varied from 10.1 °C to 14.6 °C. Second, after milling treatment, the dielectric constant (ϵ), piezoelectric strain coefficient (d_{33}), and piezoelectric stress coefficient (g_{33}) of SLS-printed PA11/BaTiO₃ nanocomposite parts were improved.

Finally, this SLS machine was used to create complex PA11/BaTiO₃ nanocomposite parts with improved dimensional accuracy and mechanical properties.

Natural polymer-based piezoelectric materials always have poor piezoelectric performance. We used TEMPO oxidized cellulose nanofibrils (TOCN) (Song et al. 2022b) and MoS₂ nanosheets to create flexible porous piezoelectric air gel films. Air gel membranes have mesopores and large specific surface areas. Moreover, using an electric field strength of 20 MV/m it polarizes the air inside the MoS₂ nanosheets and mesopores, showing better piezoelectric properties. The TOCN/MoS₂ air gel film PENG with 6 wt% MoS₂ demonstrated good power performance when arranged into a PENG. It produced a maximum volumetric power density of 0.143 $\mu\text{W}/\text{cm}$, an open-circuit voltage of 42 V, a short-circuit current of 1.1 μA , and a maximum areal power density of 1.29 $\mu\text{W}/\text{cm}$. These properties make them promising piezoelectric materials for harvesting energy.

Ferroelectric antimony sulfide (SbSI) nanowires are used to create composite materials for the fabrication of energy harvesting and sensor devices. SbSI is an excellent material for nanogenerators and nanosensors that require high piezoelectric coefficients ($d_{33} = 650 \text{ pC/N}$) and electromechanical coefficients ($k_{33} = 0.9$). In these composites, cellulose and epoxy resins serve as matrix materials, with SbSI nanowires filling the matrix. A piezoelectric response to vibration was demonstrated. We then constructed a fiber-reinforced polymer piezoelectric sensor using a composite material containing epoxy resin as an element. A comparison of the piezoelectric properties of cellulose/SbSI and epoxy resin/SbSI nanocomposite is done. Subsequently, the effect of the concentration of SbSI nanowires in the fiber-reinforced polymer with detailed properties of epoxy resin/SbSI nanocomposites were detailed.

Mechanical energy is the most common available energy source, and the piezoelectric effect is widely used to harvest it. To convert mechanical energy to electrical energy, a piezoelectric generator based on ZnO nanowires is used. Different investigations such as vibrations, human body motion, acoustic waves, wind, and other mechanical energy forms have all been studied using PENGs. Different materials and nanocomposites, particularly PZT, ZnO, BaTiO₃, and numerous substances, are used to build PENGs (Sun et al. 2020). As a matrix material, these composites use epoxy resins or various types of cellulose. Mechanical energy, which is frequently derived from the piezoelectric effect, is the most commonly available energy source. To construct PENGs, various materials and their composites, particularly PZT, ZnO, BaTiO₃, and various compounds, are used. These composite materials use epoxy resins and various celluloses as matrices (Fig. 7).

Elastic and piezoelectric properties of thin-film lead calcium titanate samples' were investigated using modified atomic force microscopy techniques such as atomic force acoustic microscopy, tapping mode, and ultrasonic piezo mode (Kopycinska et al. 2003). The morphology of thin films indicates whether they are nanocrystalline, amorphous, polycrystalline, as well as having the imaging capabilities using dynamic force microscopy techniques. The relationship between the strain dynamics and polarization gradient (Csoka et al. 2012) of these obtained films was studied by monitoring their deflection with an atomic force microscope

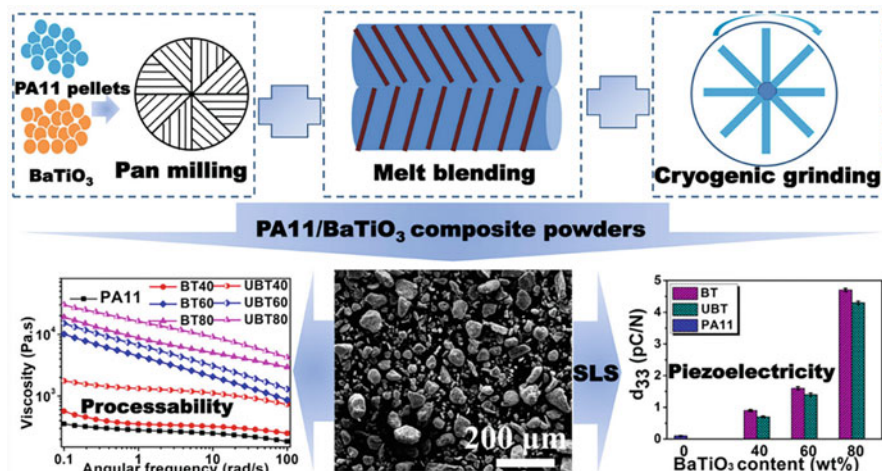


Fig. 7 Piezoelectric properties for selective laser sintering and PA11/BaTiO₃ nanocomposite powder preparation with improved processability

operating in contact mode. The piezoelectric response of the film is related to the direct investment of the cellulose asymmetric crystal structure. The effective piezoelectric shear constant (d_{25}) for highly ordered CNC films was found to be 2.1 pm/V. This value is comparable with the piezoelectric metal oxide reference film (Fig. 8).

An applied uniform strain in piezoelectric materials can induce electrical polarization and vice versa. This technical property is restricted to noncentrosymmetric systems owing to crystallographic considerations. Even in nonpiezoelectric dielectrics, non-uniform loading can break inversion symmetry and induce polarization. The key idea is that all dielectrics exhibit the above-mentioned (Sharma et al. 2007) coupling between polarization and strain gradient. The flexoelectric effect is an experimentally verified phenomenon. This flexoelectric coupling is very small and cannot be detected experimentally unless very large strain gradients are present. Based on the field theoretical framework and the related green functional solutions developed, there is an opportunity to engineer 'piezoelectricity', i.e., large strain gradients present inside composites containing nanoscale heterogeneities. Total polarization can only be achieved if the shape and distribution of the heterogeneity are not centrosymmetric. Based on restrictive assumptions about the shape, distribution of the heterogeneities and limited material data, unoptimized quantitative results show an apparent piezoelectric behavior of nearly 10% of the quartz for heterogeneity sizes within 4-nm range. Improved performance based on geometry, proper material selection, and topology optimization is not unreasonable.

Low-temperature, screen-printable piezoelectric nanocomposite films with piezoelectric and dielectric properties made on flexible plastic and textile substrates are reported. The improvement in piezoelectricity is due to the addition of silver nanoparticles (Almusallam et al. 2017) and the use of an additional post-treatment, cold pressing. These developments increased the self-supporting piezoelectric

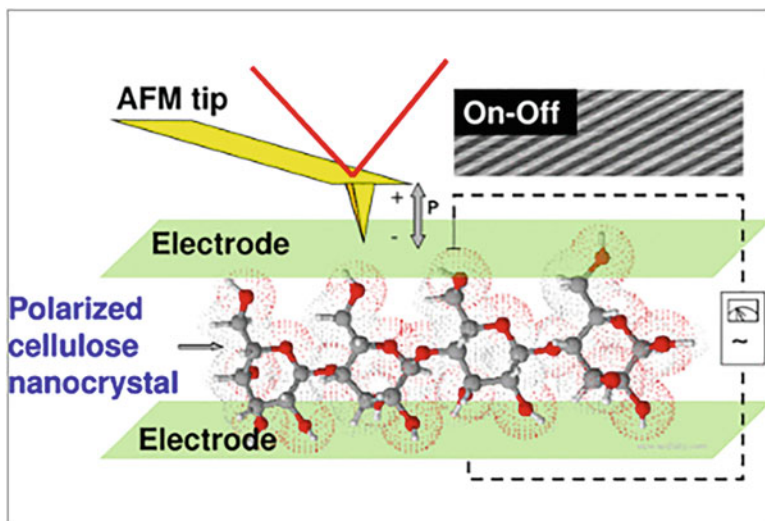


Fig. 8 Cellulose nanocrystal thin films have a piezoelectric effect

charge coefficient d_{33} by 18% to a value of 98 pC/N. The peak output voltage of the composite film decreased as the dielectric constant of the piezoelectric film changed. The theoretical and experimental potential of this material to harvest mechanical energy from various fibers under compressive and bending forces are investigated. Under 800 N compressive force, the maximum energy density of the modified piezoelectric material was determined to be 34 J/m³ for Kermel textile.

On cotton cloth, the maximum energy density of the modified piezoelectric material after bending was found to be 14.3 J/m³. For a 100-mm × 100-mm piezoelectric element with a thickness of 100 μm, this corresponds to the energy produced per mechanical motion of 14.3 μJ or 38 μJ, which is a potentially useful amount of energy. The process is illustrated briefly in Fig. 9.

Composites of MWCNT/cellulose (M/C) are formed by covalently grafting multiwalled carbon nanotubes (Yun and Kim 2011) onto cellulose. Mechanical stretching was used to create oriented M/C composites. By examining the morphology of M/C composites and measuring their electrical, mechanical, and piezoelectric properties, the strain effect was demonstrated. The effects of aligned MWCNTs on M/C composite actuator performance in terms of resonance frequency and bending displacement as a function of strain ratio and ambient humidity were investigated. Aligned MWCNTs improved mechanical and piezoelectric properties, but they also changed the actuator performance of M/C composites.

A confinement cell technique was used to produce colloidal opal structures to vertically align large CNC rods. Because of the high interfacial energy between the surface of CNC poly (tetrafluoroethylene) (PTFE) (Wang et al. 2020) and the torque induced by shear force, CNC rods are vertically aligned to a high degree. An external

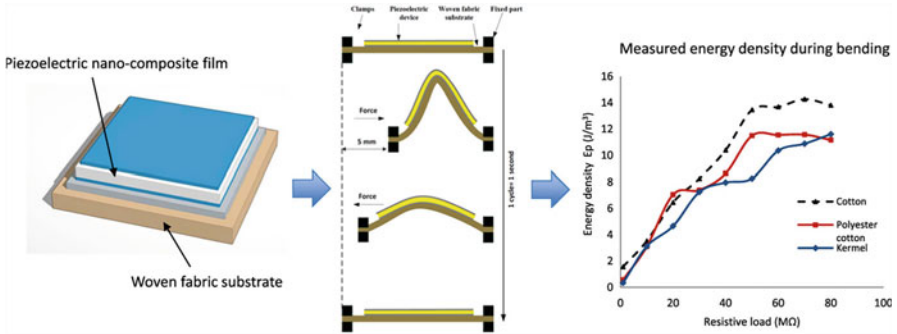


Fig. 9 Piezoelectric nanocomposite films for textile kinetic energy harvesting

DC electrical field was introduced to further align the dipole moments of each CNC in the same direction.

The CNC film with a piezoelectric coefficient of 19.3 pm/V, has excellent piezoelectric performance, which is comparable to poly (vinylidene fluoride), d33 (20–30 pm/V). This is plotted in the graph; this research introduces a new class of high-performance piezoelectric polymer materials derived from biocompatible and renewable natural resources (Fig. 10).

The frequency constants (N_p , N_t), piezoelectric voltage coefficient (g_{31}), and piezoelectric charge coefficient (d_{31}) of BNT-BT0.08 ceramic have maximum values, whereas the piezoelectric charge coefficient (d_{33}) and electromechanical coupling factor (k_p) have minimum values. ($\text{Bi}_{0.5}\text{Na}_{0.5}\text{TiO}_3$ (Cernea et al. 2012) was prepared using 5, 8, and 11 mol% BaTiO_3 (BNT-BTx; $x = 0.05, 0.08$, and 0.11) with the given sol-gel technique. It was prepared in situ using the resulting gel precursor and exhibited a microstructure composed of a crystalline perovskite and nano-sized particles. High-density (98–99% ρ_{theor}) BNT-BTx ceramics were fabricated from this nanopowder using the spark plasma sintering (SPS) technique. The results confirm the SPS method applied to the nanoscale powders obtained by sol-gel as a viable method for fabricating nanostructured ceramics. We have studied the changes in the structural and electrical properties of ceramics with BaTiO_3 concentration (x). The dielectric constant of the BNT-BT0.08 ceramic = 2090, 100 kHz) is higher than those at $x = 0.05$ ($\epsilon_r = 1350$) and $x = 0.11$ ($\epsilon_r = 1800$). BNT BT0.

The electrical properties of ceramics are affected by grain size, oxygen deficiency, and uneven residual stress caused by this oxygen deficiency. BNT-BTx ceramics sintered by SPS appear to be excellent ceramic resonators with high mechanical quality factors (Q_m). The piezoelectricity of wood, or the change in electrical polarization caused by mechanical stress (Tuukkanen and Rajala 2018), has been known for decades. Looking at an isolated crystalline wood block CNC, the piezoelectric effect is greatly enhanced. A related issue has received limited attention in the scientific literature, with little recent evidence for CNC piezoelectricity. The charge density produced by a given applied voltage is described by the piezoelectric coefficient d_{mn} . A 3×6 matrix can represent the components of a cubic tensor with

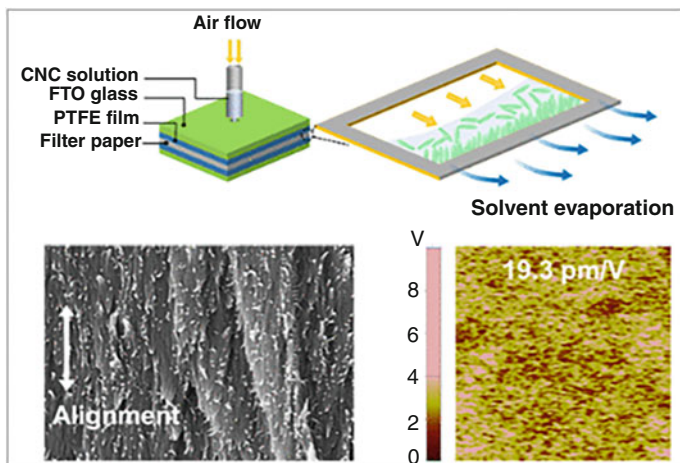


Fig. 10 Large-scale vertical crystal alignment in piezoelectric nanocellulose thin film

piezoelectric coefficients d_{mn} , where $m = 1, 2, 3$ are electrical axes and $n = 1, 2, 6$ are mechanical axes. The length, width, and thickness are represented by principal axes 1, 2, and 3 respectively, and shear around these axes is represented by indices 4, 5, and 6.

The symmetry of a cellulose crystal lattice, formed by unit cells of cellulose molecules, can be used to calculate the piezoelectric tensor d_{mn} for a single cellulose crystal ($[C_6H_{10}O_5]_n$). The cellulose has a monoclinic symmetry with a space group of $C2 \parallel x3$ and the piezoelectric tensor:

$$d_{mn} = \begin{pmatrix} 0 & 0 & 0 & d_{14} & d_{15} & 0 \\ 0 & 0 & 0 & d_{24} & d_{25} & 0 \\ d_{31} & d_{32} & d_{33} & 0 & 0 & d_{36} \end{pmatrix} \quad (E1)$$

It should be noted that this tensor is only valid for single-cellulose crystals and randomly oriented crystal arrangements, such as in cellulose nanofiber (CNF) films. Combining different coefficient yields the overall piezoelectric response. Aggregation of fibers and associated cellulose crystals, for example, has been shown to significantly reduce the effective piezoelectric tensor in wood.

$$d_{mn} = \begin{pmatrix} 0 & 0 & 0 & d_{14} & 0 & 0 \\ 0 & 0 & 0 & 0 & d_{25} & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \quad (E2)$$

where $d_{14} = -d_{25}$.

As piezoelectricity is closely related to ferroelectricity, we also measured the ferroelectric hysteresis of the CNF films using a ferroelectric tester. The piezoelectric

sensitivity of the sensor was determined by applying a dynamic excitation force (1.4 N peak-to-peak; frequency 2 Hz) to the sensor while it was resting on a metal plate (Hänninen et al. 2016). A commercially available, highly sensitive dynamic force sensor was used as a reference for the excitation force. The measurements were repeated three times per side, and each sensor was excited six times. After each measurement, the sensor was repositioned on the metal plate. Piezoelectric sensitivity (in pC/N) was obtained by dividing the charge generated by the sensor by the force of the dynamic force sensor.

The piezoelectric behavior is highly dependent on the chosen combination of nanocellulose and chitosan. In particular, chitosan and CNC can be mixed to produce films with strong lateral dependence. This effect was not seen in the chitosan + CNF films with the minimal piezoelectric sensitivity values. The purest chitosan produced the best results (Pongampai et al. 2021). The piezoelectric properties of various films appear to benefit a wide range of sensor and actuator applications. However, differences in sensitivity values between individual sensors were observed in all films studied, indicating the need to optimize manufacturing parameters such as pH, solution concentration, and mixture composition (Song et al. 2022a).

Piezoelectric materials can be employed as embedded sensors as well. Composite laminates are fundamental innovative construction materials. They are frequently used to assess structural strain and deformation. The effect of SbSI nanowire concentration on the properties of epoxy resin/nanowire composite (ESNC) and its fiber-reinforced polymer was also demonstrated. The observations of this previous work on the alignment of SbSI nanowires within an epoxy resin matrix during the electrical field curing process are discussed in the literature review of this work (Torón et al. 2020).

Figure 11 shows the schematic representation of sonochemical fabrication of composites of epoxy resin/SbSI nanowires, SbSI nanowires, cellulose/SbSI nanowires, fiber-reinforced polymer (FRP) laminate, and generator/sensor assembly with an integrated sensor.

A nondestructive three-point bending test was used to describe the piezoelectric properties of FRP laminate sensors. The experiments were carried out on an INSTRON 4469 machine (INSTRON, Norwood, MA, USA). The distance between the stanchions was 200 mm. The samples were bent with different speeds of load bar travel (1, 2, 5, 10, 20, 40, 60, 80, 100, 150, and 300 mm/min) up to a deflection of 1.0 mm. The sample was then flexed for 120 s before being reloaded at the same rate. After 30 s (the time required to discharge the sensor), the next charge rate charge-recharge cycle was performed (Fig. 11).

The electrical signal of the FRP laminate was recorded with a Keithley 6517A electrometer (Cleveland, OH, USA). During nondestructive deflection, load-deflection curves were recorded. Agglomeration of SbSI nanowires means less random distribution within the matrix and piezoelectric charges discharged unevenly at the electrodes through the nonconducting epoxy (or cellulose) matrix. Both ESNC and FRP laminates have a lower piezoelectric response. The mechanical response of the composite is reduced as the content of SbSI nanowires increases.

Three orientation methods were used to understand the effect of orientation (Zhai et al. 2020) on the piezoelectric properties of CNF ultrathin films in the thickness

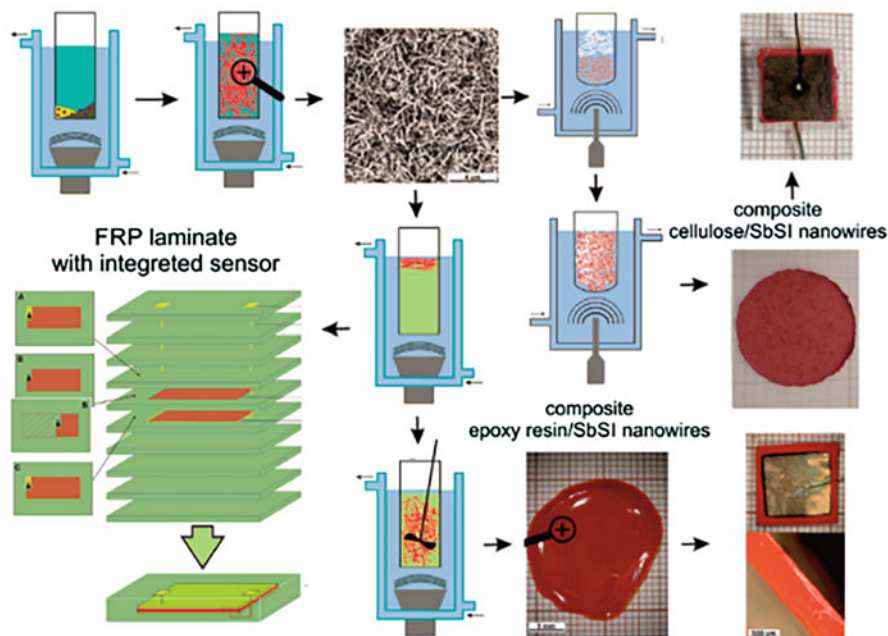


Fig. 11 Scheme of sono-chemical preparation

direction: in-plane electrical orientation, high magnetic field orientation (HiMA), and corona poling. Piezoelectric properties were analyzed using piezo response force microscopy (PFM). The vertical displacement of an extremely thin CNF film surface was captured and converted to the piezoelectric coefficient d_{33} by applying an AC voltage with PFM. The piezoelectric coefficients have different aligned CNF ultrathin films. Corona poled CNF ultrathin films exhibit the largest piezoelectric coefficient among the three orientation methods. Water contact angle measurement proves that the through-thickness piezoelectric constant is related to the hydroxyl groups appearing on the surface of the CNF ultrathin films. Quantitative PFM is generally limited by the presence of an offset in the amplitude of the piezo response. The amplitude of the piezo response should be recorded as a function of the applied AC voltage. An AC voltage up to 10 V was applied to the AFM probe with a frequency of 600 kHz and an amplitude of 2-V steps. Five different areas measuring $10 \times 10 \mu\text{m}^2$ were scanned for each sample. The sampling rate was kept at 0.5 Hz. The PFM standard sample periodically poled lithium niobate (PPLN) was obtained from Bruker and has a nominal piezoelectric coefficient of 7.5 pm/V. PPLN was used as a reference to calculate the piezoelectric coefficient of ultrathin CNF films.

Experimental conditions were similar to those described above. The amplitude signal (Aamp) obtained from the sample, is expressed in mV. Using the piezoelectric coefficients of the reference sample PPLN and equation (S1), the calibration

parameters can be determined. Once the calibration parameters are obtained, the PFM signal Aamp from the CNF ultrathin film can be used to calculate its piezoelectric coefficient d_{33} . This is because 3 represents the thickness direction and d_{33} is the piezoelectric coefficient in the thickness direction.

Yang et al. introduced BT and graphene into poly-L lactic acid (PLLA) scaffolds with the aim of imparting precise piezoelectric properties to the scaffolds. PLLA was used as a biodegradable polymer as the matrix fabric. Scaffolds were fabricated using selective laser sintering (SLS). This is a typical additive manufacturing process that can produce scaffolds with complex pore geometries and customized geometries. It was thought that a porous structure might need to provide a microenvironment for the mobile boom, whereas a personalized shape might need to fit the disease site perfectly. This can reduce bone loss during implantation. The PLLA framework had a very low open circuit voltage as well as a modern short circuit. As a result of the high piezoelectric properties of BT nanoparticles, the PLLA/BT composite scaffold provided an increased open-circuit voltage of 0.76 V and a short-circuit current of 5.1 nA. More significantly, the open-circuit voltage and fast switching are similarly increased to ~ 1.4 V and ~ 10 nA respectively, indicating stronger piezoelectricity. The overall piezoelectric performance of PLLA/BT composites was highly dependent on the polarization performance of BT nanoparticles.

Prior to polarization, the orientation of the domain names of the BT nanoparticles was disordered within the framework matrix. In this situation, the scaffold did not exhibit piezoelectric shock. Therefore, to rearrange the domain orientation of BT, polarization by an externally powered domain was required. However, the large dielectric difference between BT and polymer degraded the polarization performance of the piezoceramic. In general, the incorporated graphene creates a conducting channel for the polarization field, thereby facilitating electrical energy transfer to nearby BT nanoparticles. Under these circumstances, additional powered regions within the BT were relocated along the direction of the polarization order, resulting in enhanced piezoelectric response of the composite (Shim et al. 2022).

Cai et al. (2019) reported the application of electrospinning technology to develop piezoelectric polyhydroxybutyrate (PHB) nanocomposites. PHB is a biopolymer that is used commercially in agriculture, parcel stores, and biomedical fields. Bacterial diffusion can biosynthesize it on a large scale. Because of the high stereoregularity of the biosynthesized molecules, PHB has an excessive crystallinity (up to 75–85%). The crystal structure of PHB is an α -phase with two left-handed helical molecules made up of double axes along the crystalline state chain. The unit cell is orthorhombic: P212121-D42 with $a = 5.76$ Å, $b = 13.20$ Å, c (fiber period) = 5.96 Å. PHB is a piezoelectric biopolymer with suitable properties such as biodegradability, biocompatibility, and optical interest. Compared with PHB nanofibers, the nanofibers of PHB/MWCNT have a higher content of β -type crystals, improving the piezoelectric charge constant of PHB/MWCNT (25.7 pC/N), much higher than that of pure PHB (3.25 pC/N). For biomedical applications, mechanical interactions between cells and material surfaces are driven by cell adhesion, motility, cytoskeletal organization, cell

migration, differentiation, and tissue morphogenesis. Several studies have shown that some cell lines require external stimuli to promote tissue repair, thus the use of electromechanically active materials has attracted attention. Micro- and nanotechnology have been used to aid in the fabrication of TE scaffolds and provide a more suitable environment for potential tissue regeneration by controlling their morphology and chemistry at the micro- and nanoscale. The origin of PE was based on a non-centrosymmetric structure, and PLA had this noncentrosymmetric structure even in the amorphous state owing to its chiral conformation. For materials performing PE, displacement is produced without an external electrical field, regardless of whether there is a barrier to control the stimulation. The importance of interfaces between bioelectronic devices and the human body underscores biosecurity concerns. Biodegradability of organic and synthetic materials is one of the highly desirable properties that should be considered to avoid additional surgical intervention. A process that largely mimics biological PE is the stretching of intrinsic piezoelectric materials, which can degrade under similar body conditions. An advantage of PE in tissue regeneration is that polarized polymers exhibit more pronounced protein adsorption and improved cell adhesion and proliferation. Natural polymers are an interesting next-generation triboelectric friction material, and in recent years there has been a dramatic increase in reports of triboelectric nanogenerators based on natural polymers.

Conclusions

After referring to a wide variety of related articles the scope of cellulose nanocomposites that utilize the piezoelectric properties is obvious. In earlier times, only the applications of nanocomposites were considered based on their durability and availability; later, by considering the internal properties such as ductility, mechanical properties, and piezoelectric properties, the areas of application also changed. Now, the piezoelectric behavior of cellulose nanocomposites consists of applications in day-to-day life, biomedical applications, most particularly concentrating on health-related applications. The piezoelectric behavior of cellulose varies by layering, thickness, and components. This property can be considered catalytical, as its dimensions can vary with the constituents added to the base materials such as nanocellulose or biopolymers. This chapter illustrates the collective advances in developing a biopolymer nanocomposite for piezoelectric applications and the contributions of biopolymers with piezoelectric properties. There is a great scope of biopolymers in different practical applications, with their unique properties other than the piezoelectric effect.

Cross-References

- [Sensing and Biosensing Applications of Nanocellulose](#)

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Optical Properties of Biopolymers

9

Theoretical and Experimental Advances

Meenu Eldhose, Cincy George, Sona John, Alex Joseph, and
Liz George

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M. Eldhose · C. George (✉) · S. John · L. George
Department of Chemistry, Newman College, Thodupuzha, India

A. Joseph
Department of Chemistry, Newman College, Thodupuzha, India
Department of Chemistry, Nirmala College, Muvattupuzha, India

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Abstract

Biopolymers are receiving much attention in material science and biomedical engineering fields because of their renewable nature, anisotropic form, exceptional mechanical capabilities, high biocompatibility, tailored surface chemistry, and intriguing optical properties. The surprising inherent features of biopolymers, including chemical inertness, amphiphilicity, mechanical strength, high stiffness, and low density, allow it to be used in a broad array of optical-electronic devices. In this chapter, we focus mostly on theoretical and experimental advances of the optical properties of various biopolymers and their applications in devices like LEDs, solar cells, printed electronics, thermal transistors.

Keywords

Biopolymer · Optical properties · Nanocellulose · Bionanocomposites

Introduction

Electronic devices are getting widespread acceptance for their applications in medical field, energy harvesting, remote monitoring, etc. In the last 10 years, the convenience and value that the function devices have brought to the everyday lives of people has been remarkable.

However, the environmental problems caused by persistent materials used in electronic gadgets are increasingly severe, for example, plastics. These materials are highly stable, which will not break down in long periods without using high-temperature treatment or a harsh chemical.

Hence, academic studies of green, degradable materials to build electronics have been very significant for scientific community. In an effort to substitute for conventional resources and to minimize environmental harm caused by synthetic materials, there is an increasing desire to use commonly degradable biopolymers in electronic devices Agate et al. (2018).

Biopolymers are polymeric biological compounds in which covalent connections connect monomeric units to form bigger molecules. The word bio indicates that the materials are biodegradable and derived from living beings. Biopolymer can be used to describe a variety of materials often produced from bio sources, such as microbes, vegetables, sugar, fat, proteins, plants, and trees, among others Ahmadi et al. (2017). Biopolymers provide functional electronics with novel opportunities due to their biocompatibility, biodegradability, natural abundance, mechanical flexibility, and light weight, thereby providing the final inspiration and an alluring ingredient for the development of flexible, wearable, implantable, and/or environmentally friendly electronics.

In particular, a broad range of molecular engineering strategies has been proposed for modification of biological molecules or the utilization of molecular assemblies for achieving new optical-electrical properties, functions, and applications through noncovalent/covalent bonds with functional groups, such as hydroxyl, carboxylic, amino, and amine. Moreover, biopolymers also possess certain unique electrical properties, which motivate them to become an ideal choice for electronics devices, having high electric conductivity, good ion conductivity, versatility, and stability, etc. So far, a number of natural or synthetic biopolymers for electronics devices like batteries, PV, sensors, displays, transistors, memories, friction generators as substrates, active layers, and electrodes, give the bioelectronics the ability to be manufactured on large scales with lower costs, maintaining high performance. Moreover, the molecules of biopolymers possess an abundance of functional groups, which allows stable dispersion or high solubility in ordinary fluids, including water, for forming solvable electronic inks.

This characteristic makes them an ideal candidate to be manufactured into electronic DEVICES using low-temperature fabrication techniques, protecting them against degradation by harsh conditions from any patterning techniques like photolithography on semiconductors of organic origin. Among various fabrication techniques, solution-coating and patterning processes are the beneficial enabler technologies to develop cheap, large-area, and flexible bioelectronic devices. Recently, several common printing techniques, such as intaglio, screen, inkjet, and 3D printing, are being extensively applied to different applications, such as health care, power devices, memory, and sensors, showing high promise in developing flexible devices.

Biopolymers can be prepared using renewable materials, such as plants, crops, trees, and their components, using physical, chemical, and biological methods. Biopolymers containing natural bioactive components have outstanding features, such as high mechanical strength, biocompatibility, and biodegradability, as well as a large number of active bonding sites, which provide unique benefits for the production of flexible electronics. For instance, biopolymers' unique multilayer structures possess an adjustable stretch modulus, offering great mechanical flexibility and enhanced strength for adaptation on various dynamic surfaces and bent interfaces.

In addition, a variety of functional groups in biopolymers, such as hydroxyl, carboxylic, amino, and amine, impart diverse functionalities on electronic elements based on biologicals, including selective adsorption, sensing, and ultra-hydrophobicity. In addition, biopolymers are suitable for the creation of implantable devices because of their low cost, biodegradability, and biocompatibility. Moreover, biopolymers also possess certain unique electrical properties which make them an ideal choice for electronics devices, such as high electric conductivity, high resistance to dielectric breakdown, superior ion conductivity, etc. However, inherent limitations in the biomolecules including instability, difficulties to sustain the activity of biomolecules, and limited functionalization hamper practical applications of biomolecules for the design of bioelectronic devices Chung et al. (2019).

Unlike synthetic polymers, biopolymers are assembled by molecular means, leading to well-defined three-dimensional structures. Biopolymers are

environmentally benign in nature and are easy for the microorganisms to break down. The term “biopolymer” refers to synthetic polymers that are produced bio-based from living things. Their molecular structures are mostly made of repeated units of amino acids, sugars, or nucleic acids, along with chemical side chains to aid their functioning.

Because of growing ecological concerns stemming from the use of chemicals and products made of oil, synthesizing refined chemicals and functional materials from natural resources is a matter of great social importance. Because of their intrinsic qualities, renewable nature, and abundant nature, nanocellulose could prove one of the most promising green materials in the current era, from sourcing, production, and surface modification of nanocellulose, to materials creation and applications. Biomass, plants, or microorganisms could be used as sources for nanocellulose, using relatively simple, scalable, and efficient isolation procedures. Nanocellulose can be extracted from natural sources using a combination of mechanical, chemical, and enzymatic processes. The source, the extraction methods, and the potential subsequent surface modifications affect nanocellulose characteristics. When materials are required for material strength, flexibility, and/or nano-structured specialty, nanocellulose is a viable material. Applications include tissue scaffolds, biomedical applications, biosensing, ultracapacitors, catalysis, environmental monitoring, optical electronics, antibacterial coatings, functional papers, packaging, mechanically reinforced polymer composites, and electrochemically controlled deposition. Techniques to alter nanocellulose surfaces include esterification, amidation, polymerization, etherification, silylation, sulfonation, and phosphorylation. These methods are typically applied to introduce charge- or hydrophobic-like moieties. The optical properties of nanocellulose provide a broad design and application opportunity for optoelectronics, including solar cells (Lasrado et al. 2020), displays, flexible electronics, and touchscreens (Hu et al. 2013). Because they allow the flow of light, cellulose-based materials are naturally transparent. However, if the light is presented as fibers that are of similar sizes as light wavelengths, then they may scatter substantially. Due to its intriguing characteristics and potential applications, nanocellulose has received much attention recently as a biogenic, biorenewable material. It can be extracted from various materials that contain cellulose, of which wood is the most prevalent source. Natural wood fiber has a distinct, layered, hollow structure. The cell walls of individual wood grain are composed of numerous nanofibers. Nanocellulose fibers can be woven out of pulps made of wood using mechanical techniques like homogenizers, microfluidizers, and milling machines. Flexible electrically conducting sheets that have high surface conductivity, wide optical absorption, and outstanding chemical and thermal stability are required for optical electronics applications. Silver nanowires (AgNWs) bonded on the surfaces of hybrid membranes made from cellulose nanofibers (CNFs) and graphene oxide (GO) were created using an easy vacuum-filtering technique, layer-by-layer, and were developed by Changshun Wang and collaborators (Wang et al. 2020). The AgNWs layers are capable of developing condensed, Stratiform structures based on a flexible, robust base provided by intermediate CNF/GO layers. The measurement of light in nanocellulose and its optical properties with respect to the dielectric

materials are covered in this chapter. The chapter also covers materials based on nanocellulose for environmental protection, plasmonics, photoreduction, and in energy-saving buildings and systems. Furthermore, it offers opportunities to develop cutting-edge strategies to build unique materials based on nanocellulose, which possess different dimensions and have novel applications. Lightweight, flexible supercapacitors are the most important potential optical-electronic application for nanocellulosic materials. When combined with conductive substance – typically a polymer – nanocellulose provides requisite mechanical support for flexing, freely standing structures and produces a large volumetric capacity. Modifications to nanocelluloses optoelectronic properties can be made through modifications to conductive polymers. Polypyrrole, polyaniline, and polycarbonate are the most commonly used conductive polymers. Polypyrrole has been applied on to the fibers of cellulose in order to produce a CNF-based electroactive composite, created by Nystrom et al. This composite is not only electroactive, it is also conductive as well as having power storage capabilities. The enhanced polypyrrole/nanocellulose composite produced an almost perfect pseudocapacitive response.

Bionanocomposites

Using conventional chemical methods, like polylactic acid, or by direct enzymatic action within a microorganism or a genetically modified organism, like polyhydroxyalkanoate, a biopolymer could be created based on biomonomers. The biotechnological creation of biopolymers with customized properties that are suitable for high-value medical applications, such as tissue engineering and medication delivery, is enabled via genetic modification of microorganisms.

Biopolymers have been used by humans for the most part throughout history in the form of food, clothes, and furniture. Since the start of the Industrial Revolution, fossil fuels like petroleum have been the most prominent sources for the invention and production of virtually all commercial products, especially plastics, now in widespread use (Sundar et al. 2010). However, these resources are finite, and ecological considerations have to be taken into account for all aspects of the usage of fossil fuels in manufacturing and power. Biopolymers need to compete against fossil-fuel-derived polymers, not only based on function, but also on price. Biopolymers are competitive in this respect when oil prices are high, while the prices of biomass, like maize starch, are low.

Bionanocomposites are worthy of a specific investigation, since they constitute an exciting multidisciplinary field combining biology, materials science, and nanotechnology. Polymer nanocomposites are composed of polymers and inorganic/organic fillers, which are combined on the nanoscale. The broad array of available biopolymers and fillers, such as clays, cellulose whittling, and metallic nanoparticles, gives these new materials exceptional tailorability. Nanocomposites are pursued by biomedical technologies such as tissue engineering, medical implants, dental uses, and controlled drug delivery.

However, widespread application of these significant bionanocomposites in our everyday lives is achievable only when it is available to consumers in bulk. Biopolymers or bionanocomposite is biocompatible as well as biodegradable, which is readily absorbed or eliminated by metabolic processes from the body. Biopolymers could offer competitive advantage in replacing petroleum-based polymer materials sustainably. They also possess unique characteristics, which cannot be replicated with conventional polymers. Biopolymer properties are strongly affected by polymer structure, which is crucial in producing polymers for their intended uses.

Biopolymers are used in a wide variety of medical applications. Packaging, engineering, environment, agriculture, and food-related industries are also seeing an increase. Adding porosity to a biomaterial broadens its possible applications. Biopolymers and their metabolites are abundant, diverse, and essential for living things; they possess unique characteristics that make them most useful for various applications. Biopolymers are a good choice for industrial applications due to their characteristics and because they can be made from renewable resources.

When one compares the performance properties and manufacturing costs of biopolymers with those of fossil fuel-based equivalents, the situation is one of the perfect competitions. Although it is quite cheap to manufacture biopolymers, continuous efforts are being made to improve their beneficial properties. Recently, a wide range of biopolymer applications have been identified, including additives and bioplastic blends, as well as personal care, food, and pharmaceutical goods, all with an eco-friendly degrading benefit (Sadasivuni et al. 2019).

According to K. Winkworth-Smith and T. J. Foster (2013), the target for a chosen biopolymer (emulsion-and-foam-surface activity, viscosity formation, or gelation), its structural development process, and its collaborative efforts with any complementary building materials play roles in the determination of biopolymers function in different fields. Two types of biopolymers are biodegradable and non-biodegradable. The above groups emphasize on the importance of conducting a comprehensive study on biopolymers, because this is necessary for developing eminent products, which can safely be biodegraded.

This has enabled the development of biocomposites, which are essentially polymers created from biologically based or fossil-fuel-based components reinforced with natural fibers such as flax, hemp, sisal, wood, and jute, thereby improving the biopolymer's composition. Biocomposites have generated enormous interest in the current research marketplace, providing the means for the resolution of a number of obstacles while trying to produce new products S.J.Eichhorn et al. (2020).

Classification of Biopolymers

Biopolymers are divided into two categories according to their sources of origin: natural – derived from plants, animals, and microorganisms, or fossil fuels – and synthetic – derived through the chemical process of synthesis. When measured on the basis of their usability, nondegradable biobase biopolymers are superior to biobase-degradable biopolymers on different functional fields (Ravenstijn 2010).

Biopolymers are classified as elastic, thermoset, or thermoplastic depending on their capacity to react under thermal conditions. Elastomers motivated many scientists to create new materials that possess their own set of desired properties, while thermosets from biological sources recently have attracted greater interest for manufacturing components than bioplastics from biological sources.

Biopolymers may also be classified as mixtures, laminates, or composites depending on their composition. Many biopolymers have demonstrated their ability to be blended with another biopolymer, whether biodegradable or nonbiodegradable, either natural or synthetically generated (Vroman and Tiggert 2009). Several biopolymers have proven the ability to be blended with another biopolymer, either biodegradable or nonbiodegradable, naturally or synthetically generated.

Additionally, biopolymers are categorized based on the monomeric units used and the structure of biopolymer forms. DNA and RNA are longer polymers made up of at least 13 nucleotide monomers.

Electronic Fabrication Techniques Using Biopolymers

Biopolymers are susceptible to degrade in the presence of high-light, heat, and chemical corrosion, and therefore, conventional top-down photolithography and thermal vaporization are ill-suited for the fabrication of electronic devices made of biopolymers because of the dissolution in the washing process with organic/acid solvents, or the warping in the thermal processing. Printing is an additive technology that has the capability to deposit various materials simultaneously and pattern them to produce flexible devices like medical monitors, human-machine interfaces, electronic displays, transistors at a low cost, high volume, and high yield. Biopolymers are not dissoluble in organic, acidic, or aqueous solutions, so they may be applied to solution-processing printing processes. Through printing procedures, biopolymer inks can be easily converted into functional electronics without the need for severe chemical or thermal processing, thereby preventing the loss of bioactive components and enabling the production of stable, high-performance electronics.

In addition, printing is one of the most promising technologies for processing solutions, covering both contact printing and contactless printing. As with screen and gravure printing, contact printing requires a mask to be in contact with a substrate, restricting its applicability to curved or complex surfaces. In contrast, contactless printing eliminates the need for masks and enables the direct printing of various surfaces. Therefore, depending on the surface qualities, the precise biopolymer ink patterns and high-performance electronics can be printed using the most suitable methods.

Screen Printing

Screen printing is a printing process in which ink is applied onto a substrate by pushing a viscous, high-viscosity ink across a screen in the shape of a pattern. In recent years, screen printing has received considerable attention for the printing of

photoelectric devices such as printed solar cells, organic light-emitting diodes, organic field-effect transistors, and radio frequency tags. For example, PEDOT:PSS printed by screens onto the electrode surfaces has been proven an efficient conductor and redox mediator for biosensors, which has a high sensitivity for the detection of phenolic compounds in water. Furthermore, the screen-printed PEDOT:PSS circuits on fabrics have shown a high sensitivity for the electrocardiography signals as an electronic and an ionic conductor.

As expected, screen-printed polyfunctional electronics textiles, which have intrinsic biocompatibility and flexibility, have a high potential of application to the smart wearable electronics of the future. Therefore, the screen-printing technologies are extremely promising for the production of biopolymer-based electronics at a lower cost and with less impact on the environment.

Spray Printing

At the moment, the spin-coating technique is still one of the important methods for the process to shape solution films. However, the spin-coating technique has very high requirements to fit surface characteristics of rotating materials to substrate surfaces.

In addition, a poor utilization rate and nonuniform films restrict its applications to devices with a lower price and a greater yield. Spray-coating has been extensively utilized to flexible electron devices because of its improved compatibility with biopolymers, high-efficiency manufacturing, and lower-cost manufacturing procedures in order to address the disadvantages of spin-coating. For instance, it is compatible with the mass production of the industrial powder, for instance, with large-scale fabrication of biopolymer electronics films by rolling-to-roll.

Inkjet Printing

Currently, the biopolymer films coated by aerosol spray are being extensively studied for electronics. In brief, there are big prospects in spray-coating for the field of integrated circuits at scale. As a contactless technology, inkjet printing works by dropping an ink gradually onto the substrate, to create a patterned predesigned film, and has been used extensively for fabricating a wide variety of devices such as transistors, solar cells, and sensors S.Conti et al. (2020). Unlike the above two print methods, inkjet printing is capable of producing highly resolved prints of electronic functional biopolymer inks onto different substrates, showing promise in fabricating micrometer-scale devices with custom patterns.

By using inkjet printing, the electrode structures can be freely inked into any geometry, a significant improvement over conventional processes like as photolithography or thermal vaporization via shadowmask. In addition, inkjet printing is suitable for fabricating thin, homogeneous layers at moderate processing temperatures that are compatible with biopolymers and flexible biopolymer substrates. Inkjet

printing is thus a highly promising route to build complex, inexpensive electronic devices with biopolymers.

3D Printing

As a significant advanced additive manufacturing technology, 3D printing is capable of producing accurate complex 3D architectures with no material waste to fulfill a variety of practical applications. Moreover, 3D printing can be used for biopolymer-based inks, avoiding damage to biological activity, and importation of toxic materials, to produce biomedical devices and nonplantable electronics J.Ferri et al. 2019. Therefore, introducing 3D printing in smart materials and biopolymers becomes crucial for the further expansion of 3D-flexible electronics for tissue engineering, soft robotics, and stretchable electronics.

Moreover, 3D printing is utilized extensively in tissue engineering, thermonuclear delivery, and other biological applications. Thus, 3D printing provides advantages such as superior print flexibility, biocompatibility, and environmental friendliness, allowing it to be applied to a variety of biobased electrical devices. Great advances in biopolymer-based electronics have been achieved in preparation of printed methods.

When considering to select the most suitable technology, following issues are addressed, depending on biopolymer inks and fabrication technique: biopolymers for printing that have the right rheological properties, such as viscosity, concentration, and plasticity of the biopolymer inks, as well as the compatibility of printing technology with biopolymers, to prevent the loss of bioactive components and the corresponding performances. In addition, the wetting and dewetting kinetic mechanisms of biopolymer inks on various substrates are not entirely known, necessitating additional research to achieve regulated and precise patterning using biopolymer inks. Therefore, greater emphasis must be placed on optimizing the printing methods depending on different biopolymer inks, which is crucial for realizing biopolymer applications for electronics.

Application of Biopolymers

Due to characteristics such as biocompatibility, degradation by microbes into non-toxic end products, maximization of biological activities, lower immunogenicity, ability to support cell proliferation with the proper mechanical properties, transposition into complex shapes with the appropriate porosity, and preservation, biopolymers are utilized most frequently in the medical field. Examples include targeted drug delivery, surgical stent devices, wound healing products, and closed-end products. Biopolymers are generally used in applications such as food containers, soil holding sheets, agricultural films, garbage bags, and common packaging materials because of their film-forming and barrier properties. They are also found applications in hazardous waste disposal, automobile material production, the paper industry, and even the development of new construction materials.

Three-dimensional scaffolds to form tissues, packaging, delivery systems, environmentally friendly insulation materials are some of these applications (Gavillon and Budtova 2007). Biomedical applications, due to the higher importance of their entities, attract much of the interest in biopolymer applications in comparison with other fields. The most common applications for biodegradable polymers in disease treatment are orthopedic and vascular reconstructive implants and surgical sutures that aid in the integration of damaged tissues.

These threads may work throughout tissue regeneration, before being either physically removed or allowed to disintegrate naturally within the body. They are also highly durable, easily sterilized, and strong. Because of their stable tensile strength and outstanding flexibility, poly-(glycolic acid), poly-(l-lactic acid), and their copolymers are commonly used as stitches. Bone fixation implants made from biopolymers allow unrestricted, dynamic bone regeneration, and they do not have to be removed by surgery once bone has healed, as the whole implant structure disintegrates.

Because they are highly bioactive, metabolic, and toxic when compared with their synthetic counterparts, biopolymer nanoparticles potentially become an essential material in drug delivery systems and facilitate the release and uptake of drugs. Biopolymers having poly-(hydroxy acids) and poly-(ortho esters) groups are usually employed for drug delivery systems. Polyurethanes are classified as a blood-compatible material due to their hard, flexible, and wear-resistant properties, which are essential in scaffolds of blood vessels simulations by the artificial grafts (Zhu and Xu 2010). Biopolymers have attracted interest from the agriculture industry because of their nature-based degradation and soil-improvement properties, with significant potential for the development of mulches and plant containers.

By maintaining moisture, decreasing the weed growth, maintaining proper soil temperature, and – most importantly – photodegrading, organic fertilizers contribute to plant growth and remove the need for removing them and associated costs (Raj et al. 2011). Fertilizers are created using polymer films comprised of poly(vinyl chloride), low-density polyethylene, poly(vinyl alcohol), or polybutylene. With the help of soil microbes, or by adding fine particles that promote the films termination, these films are modified so they may begin breaking down only after the growing season has ended. In farm settings, composting lessens the need to farm, meaning that root injuries and plant stunting/destruction are reduced further.

Because of their physical characteristics that enable modifications of molecular weight, chemical composition, and manufacturing conditions, yet achieving desired properties, packing polymers are of great interest. These desired properties are determined by the product to be packaged and its storage environment. The possibility to combine properties from several biopolymers in one biodegradable film has been established in order to create packing materials that possess features quite notable compared with synthetic equivalents, although the latter do enjoy some advantages compared to their bioderived counterparts.

Potential packaging films are being created using polyethylene copolymers and biopolymers based on starch, cellulose, and other polysaccharides. Natural fillers, such as starch, are now mixed with synthetic polymers in order to enhance the degradation-free qualities of the packaging laminates. Food-storage bags, storage

containers, throwaway plates, glasses, and silverware, and even textiles are among the preferred types of packaging that are made with biopolymer sources. Biopolymer packaging of foods and condiments assures the quality and appetizing qualities of packaged items are maintained from production time until sampling.

The automobile industry has seen a major shift to produce today's cars, focusing on reducing fuel use and emissions. The forgone goals were supposed to be achieved by making vehicles lighter to meet motoring laws. Most manufacturers are racing towards producing plastic car components made from bio-derived polymers like polyamide, polyester, and polypropylene, among others.

Scale	Methods	Variables	Outcomes	Limitations
Quantum scale	Quantum mechanics (QM) Density functional theory (DFT), Coupled cluster method (CCSD(T))	Crystal symmetries	Thermal properties, chemical reactions, and electrical qualities based on band structure and density of states	Timescale and system size constraints imposed by computing performance.
Mesoscale	Coarse-grained methods. Quasicontinuum method, Kinetic Monte-Carlo method, dissipative particle dynamics (DPD), coarse-grained molecular dynamics	Structure flaws and defects, molecular weight, thermal fluctuation, hydrophilic-hydrophobic – ratio	Hydrodynamic radius, fracture properties, radius of gyration, self-assembly and size effects	Either restrictions in accuracy or size
Molecular scale	Energy conservation and equilibrium, steered molecular dynamics (SMD), replicate exchange molecular dynamics (REMD), molecular dynamics (MD), well-tempered metadynamics, umbrella sampling, and reactive force-fields	Sequence, pH, ions, force, velocity, temperature	Conformation, chemical reactions, mechanical properties	Restrictions in time scale and system size due to constraints in computational performance

(continued)

Scale	Methods	Variables	Outcomes	Limitations
Macroscale	Continuum methods such as solid and CFD (FEM, FVM, BEM, FDM) MBS-simulation	Process conditions (thermal, mechanical, and electrical), the global structure, and dynamic mechanical properties	Global thermal and electrical analysis, multibody problems, and nonlinear structure analysis	Description via partial differential equations, the restriction which arises due to homogenization of structure or properties

We review the current state-of-the-art of biopolymer design, highlighting materials-design approaches combining experimental and computational studies. Exploring a variety of parameters effects on biopolymer synthesis and processing, we argue, provides opportunities for developing mechanistic models and exploring design spaces for novel materials. As such, we purposely excluded studies which focus solely on structure analysis and the characterization of natural biomaterials.

This research begins by reviewing the general methods for the manufacture, processing, and modeling of biopolymers, emphasizing what parameters may be handled or studied using these methods. Then, we examine the structural-to-processing-property relationships for collagen, elastic, and silk-inspired biopolymers, as well as the methodology employed to arrive at these conclusions. Combining experimental and computational investigations, we then discuss challenges and opportunities in the field of biopolymer design.

Biopolymers in Biomedical Applications

Collagen

Collagen, which was discovered by Paine in 1838, is a significant structural component of vertebrates and the most prevalent mammalian protein, comprising 20–30% of the total body protein. Collagen is produced by fibroblasts, which are normally derived from pluripotent adventitial cells or reticulum cells.

The primary collagen molecules are rod-shaped, with an approximate length and breadth of 3000 and 15Å, respectively, and a molecular mass of roughly 300 KDA. It is easily absorbed by the body and possesses a negligible antigenicity. In addition, the material is nontoxic, biocompatible, and biodegradable. It possesses a high ratio of strength to weight and a high affinity for water.

Gelatin

Gelatin is a water-soluble substance derived from the degradation of the tertiary, secondary, and to some extent primary structures of natural collagen via the partial hydrolysis of collagens extracted from animal skin, white connective tissue, and

bones. The majority of hydrocolloids are polysaccharides, whereas gelatin is a protein that is edible and contains all essential amino acids with the exception of tryptophan. Gelatin can be derived from a variety of collagen sources. The primary commercial sources are cattle bones, skins, pig hides, and fish. Due to its surface-active qualities, gelatin is utilized as a foaming, emulsifying, and moistening agent in food, pharmaceutical, medicinal, and technical applications.

Chitosan

Chitosan is generated from chitin, a polymer found in nature. Chitin was originally isolated and characterized in 1811 by French scientist Henri Braconnot from mushrooms. It comes second in the world's most abundant biopolymer. Other than cellulose, chitin is the most prevalent polysaccharide in nature. It is a major component of crustacean and insect exoskeletons, as well as the cell walls of nematodes, yeasts, and fungi. Chitin is superb for biocompatibility; it has high bioactivity, biodegradability, selective permeability, polyelectrolyte activity, antimicrobial activity, the capacity for forming gels and films, and the ability for chelation ability and absorption capability. Chitosan can be used as a pharmaceutical carrier to manage drug release, to act antibacterially and antiacidic, to inhibit bacterial plaque formation and the decalcification of dental enamel, to contribute to osteogenesis, to absorb fat, and to promote healing of ulcers and lesions due to its unique properties.

Cellulose

It is the most common naturally occurring glucose polymer, being the primary component of plants and natural fibers such as cotton and flax. Additionally, some bacteria (such as *Acetobacter xylinum*) may manufacture cellulose. Chemically, plant-based cellulose is identical to that of microorganisms and bacteria; yet, its macromolecular structure and physical properties are distinct. Cellulosic fiber has the chemical formula $(C_6H_{10}O_5)_n$ and is a polysaccharide with a linear chain consisting of several hundred to over 10,000 D-glucose units linked by B(1–4) bonds. Cellulose and its derivatives are harmless to the environment. Natural cotton fibers and other recycled (man-made) celluloid fibers, such as viscose, modatim, and lyocell, are the most important celluloid fibers for medical purposes.

Xylan

Xylan, a naturally abundant hemicellulose, is regarded to be the second most prevalent polysaccharide, behind cellulose. Xylans are a predominant hemicellulose in hardwoods, as well as annual plants and grains, constituting up to 30% of cell wall material and 25–35% of lignocellulosic materials. Because it is biodegraded by enzymes produced by colonic bacteria, xylan has been deemed a promising

feedstock for the production of gastrointestinal drug delivery systems. Xylan's physiological effects: The viscous structure of fiber polysaccharides in grains contributes to the bulking effect of feces as well as reductions in blood cholesterol levels, postprandial glucose, and insulin responses. Cereals' water-extractable polysaccharides are said to alleviate alcohol-induced liver problems. Arabino-glucuronoxylan, a substance with immunostimulating properties, has been isolated from various plant sources. Several of these xylans have demonstrated antiphlogistic properties. Hemicellulose extracts rich in xylans from plant residues, such as bamboo leaves, wheat straw, and maize stalks, have been shown to inhibit the growth of sarcomas¹⁸⁰ and other tumors, most likely by the indirect, nonspecific stimulation of the host's immunological responses. T cells and immunological cells are stimulated by carboxymethylated hemicelluloses derived from wood.

Alginate

Alginate is a water-soluble polymer derived from brown algae, one of the most abundant biosynthesized substances. Alginate is utilized to support tissue healing and regeneration matrix or delivery systems. Due to its biocompatibility, biodegradability, nonantigenity, and chelating ability, alginate is widely employed in a variety of biomedical applications, such as tissue engineering, drug delivery, and, in particular formulations, to avoid gastrointestinal reflux. It is a U.S. Food and Drug Administration (FDA)-approved polymer; thus, alginate has emerged as one of the most prominent biomaterials in regenerative medicine, nutritional supplements, and other uses. Due to its easiness of manipulation, alginate is also often employed in dental impression production.

Carrageenan

Carrageenan is extracted from various species of rhodophyte marine algae. Carrageenan is the generic name for a family of polysaccharides with a gel-like consistency and viscosity. Carrageenan is a sulfated polygalactan whose ester-sulfate content ranges from 15% to 40%. Carrageenan is regarded as a suitable alternative for gelatine (products derived from animals) in both solid and soft-gel capsules. Its addition to glycerin-water mixes conceals the chalkiness of antiacidic gels. It can be applied topically as well as sublingually. It functions as a thickening and binder agent in dental products and keeps the liquid and solid from separating.

Gum Arabic

Gum arabic is a dry, edible, gelatinous substance obtained from the stems and branches of *Acacia senegal* and *Acacia seyal*, which are rich in soluble nonviscous fiber. GA is used topically to cover inflammatory surfaces and orally to alleviate

intestinal mucosal inflammation in folk medicine. According to studies, GA possesses antioxidant and nephroprotective effects. Nevertheless, these results are not universally recognized. Due to its physical qualities, it reduces glucose uptake, increases the mass of feces and Bile acids, and has the ability to modify human physiology in a beneficial manner. Some research has shown that gum arabic inhibits the growth of some pathogenic species, such as *Porphyromonas gingivalis* and *Prevotella intermedia* (agents implicated in the creation of dental plaque). According to one study, GA can promote tooth remineralization and reduce plaque production, making it a viable agent for preventing the formation of plaque. These effects were linked to the high Ca^{+2} , Mg^{+2} , and K^{+} salt content of the polysaccharides of GA, as well as the effects of the gum on the metabolism of Ca, and maybe phosphate as well. Adding gum arabic to plaster goods increases their stiffness. Small amounts of gum arabic added to hemihydrate calcium carbonate lower the quantity of water required to combine plasters and stones.

Xanthan Gum

Xanthan gum is a naturally occurring polymer generated by *Xanthomonas campestris* bacteria. Repeated pentasaccharide units consisting of two D-glucopyranosyl units, two D-mannopyranosyl units, and one D-glucopyranosyluronic unit make up the fundamental structure of xanthan. Viscosity is correlated with the ability of xanthan to thicken liquids; higher viscosity resists flow. The viscosity of xanthan solutions decreases as shear speed increases, exhibiting a pseudoplastic or shear-thinning feature. In toothpastes and cosmetics, xanthan gum is a frequent ingredient. It can be easily emptied from the tube. Additionally, it ensures that toothpaste remains stable when placed on a toothbrush. Additionally, the thinning properties of shears improve dispersion onto teeth and rinse off. Denser toothpastes containing xanthan gum have a brilliant, glossy, stringy appearance and a reduced flow rate. In pharmaceutical emulsions and suspensions, xanthan gum prevents the separation of insoluble components.

Pectin

Pectin is a heteropolysaccharide structural component of the main cell walls of terrestrial plants. Pectin consists of a complex collection of polysaccharides found in the majority of primary cell walls and is particularly common in the nonwoody sections of nearly all terrestrial plants. Commercial pectin is nearly exclusively obtained from citrus peels or apple pomace, both of which are byproducts of fruit juice manufacture. Pectins are believed to be mostly made of D-galacturonic acid (GalA) units linked into chains by A-(1–4) glycosidic bonds. Combined with carbopol and chitosan, pectin is used to generate mucoadhesive patches. It exerts its effect by making close contact with tissues, enveloping polymer-mucin chains, and forming a weak connection between the chains. Pectin functions as a natural

preventative against cation toxicity. Pectin is efficient at eliminating lead and mercury from the respiratory and digestive systems. Pectin improves blood coagulation; hence, it is useful for hemostasis regulation. Pectin is useful in the treatment of overeating because it slows digestion by keeping food components in the intestines, resulting in less food absorption. Pectin hydrogels are employed as binders in tablet formulations and in controlled-release matrix tablet formulations. Pectin is currently being studied as a material for the development of medication delivery systems tailored to the gastrointestinal tract. Some pectins have demonstrated *in vitro* anti-inflammatory capabilities, which is a promising and advantageous property for device modification. Due to its ability to stimulate immunological responses *in vitro*, pectin is currently being intensively studied as a potential candidate in soft and hard tissue engineering as well as dental titanium coatings.

Factors Affecting the Optical Properties of Biopolymers

Biopolymers have attracted much interest as an ingredient for flexible display devices. Flat-panel display (FPD) technology is moving away from a wet-bulk process towards a continuous roll-to-roll due to manufacturing cost considerations.

The traditional technology involves stacking onto a large substrate, usually made from glass, then, slicing up the panels to different sizes, depending on available tools. So, a normal way to manufacture the panes into different sizes proved to be most economical.

Plastics were used instead of glass for the substrates in the rolling-to-roll process, due to their flexibility. When the process is not being used, the plastics flexibility allows it to be bent over and folded over to store. As a result, a rolling-to-rolling approach has a number of advantages, such as cost-effectiveness and simplicity. Coefficient of Linear Thermal Expansion (CTE) is an important element of a roll-to-roll process.

If a substrate is not capable of withstanding the high temperatures of the process, then functional materials that are deposited on substrates will suffer damage and destruction due to a mismatch of material CTE. Even though the CTE of glass used as the substrate is sufficient to withstand the temperatures in the manufacturing process, the glass is impossibly brittle and cannot fold. Plastic bracing needs to be used in order to improve CTE.

When reinforcing the plastic, this reduces the plastics CTE, but does not lose optical transmission. The reduced CTE associated with nanocellulose offsets higher CTE from the polymers. On the other hand, the high Youngs modulus associated with nanocellulose is balanced out by low Youngs moduli associated with polymers.

As crystallinity increases, the intermolecular hydrogen bonding among molecular chains in the cellulose increases. Because thermal expansion is constrained by the intermolecular hydrogen bonds, higher degrees of crystallinity indicate lower CTE. Increased milling causes a degradation and breakdown of the fibrillated fibers as multiple passes are made through the mill. In conclusion, CTE and the material modulus are affected by the level of crystallinity. Furthermore, since cellulose

contains nanofibers which are connected through hydrogen bonds among the intermolecular bonds, these nanofibers are hard to disassemble using mechanical processes such as milling, homogenization, etc. So using blender-style homogenizers, they produced the nanofibers of the TEMPO-oxidized cellulose (TOCN). The CTE of TOCN (2.7 ppm K^{-1}) was lower than that of glass (about 9 ppm K^{-1}) and compared to silicon (3 ppm K^{-1}).

The nanofibers are denser, with smaller pores within fibers, suppressing light-scattering properties, while the bacteria nanocellulose is translucent to light-scattering at the surface. The nanocellulose is treated with emery paper for increasing transparency.

The surface of the nanocellulose needs to be smoothed uniformly in order to enhance light surface scattering. There are different methods to smooth the nanocellulose surfaces. The earliest technique involves coating sheets of nanocellulose with optically transparent polymers. Impregnation into a plastic has been the second method and has been mostly used.

The optical transparency of nanocomposites made from nanocellulose and plastics is enhanced because nanocellulose has smaller diameters than visible light wavelengths. The difference between the refractive index (RI) of nanocellulose and plastics is minimized in order to improve light transmission in the nanocomposites. Chemical processing changes the RI of nanocellulose. Therefore, careful management of the optical transparencies of CTEs and substrates is essential, with acetylation being the most prevalent chemical treatment.

Also, nanocomposites that contain high-DS BCs exhibit superior transmission, as BCs have lower RIs as the DS increases. Furthermore, because of the enhanced bonds acetylation created between the cellulose and the polymer, the acetylated BC was successfully incorporated. Insertion of the large groups of acetyl into nanocellulose caused a thickness increase in the material, allowing thickened nanocellulose to interact with transduction. Furthermore, excessively acetylated nanocellulose will have lower RIs compared with that of plastics. The adaptability of nanocellulose supports has several advantages, including the ease of manufacturing and storage.

The bendable, flexible substrates show lower modulus and higher extension when broken than the nanocellulose, which shows the adaptability of the nanocomposites offered by plastics. Pure acrylic resin has a very high elongation at break; however, the material is susceptible to even a small crack; hence, sheet breaks at folding. In nanocomposites, the nanocellulose (BC) stops the growth of fractures; thus, the material can fold without breaking. However, if used in dry BC, elongation at breaking increases, reducing the strength and increasing the Young's modulus. The properties of BC-containing nanocomposites are also affected by plasticization methods.

The earliest technique for introduction is "in situ" technique, involving plastic addition directly to BC development environment. The second technique of introduction involves the "impregnating" of the BC in the plastic solution. The nanocelluloses tapered structure is different depending on the nanocomposite creation process. The hydrogen bonding among the nanofibers is broken off when the PVA is coated onto BC surfaces, increasing elongation upon breaking when compared with uncoated BC.

The Young Modulus and the strain at the yield, which is affected by the number of passes in the mill, were both affected for the MFC. Young's modulus and yield strain are reduced by making more passes. These results suggested the linkage of intermolecular hydrogen bonds with the fibrillated fibers at the number of passes.

The fiber form is a source of reduced strain at the yield. First, additional grinding treatments caused fibrillated fibers to break down, as well as increasing hydrogen bonding. Second, aspect ratios of fibers decreased due to the shear forces reducing fiber length.

Optical Applications of Biopolymers

Optical components are utilized for light transmission, detection, and conversion. They include optically active coatings like sensors, photonic coatings, Bragg reflectors, and several other devices, as well as waveguides (a physical structure that conducts electromagnetic waves of the optical spectrum). The materials used to create optical elements must have the ability to modulate or control electromagnetic radiation in the UV, visible, and IR spectral ranges. Materials must possess specific mechanical, chemical, and biological (biocompatibility, biodegradability) qualities in addition to optical properties in order to be used in photonics. The degree of transparency, refractive index, and their spectrum dependence are the most crucial factors in choosing an optical material. High transparency materials exhibit comparatively little light reflection, absorption, and scattering, resulting in minimal optical losses. However, biopolymers substantially absorb light at short wavelengths of 250–400 nm, which is common for many organic compounds. As a result, the optical use of materials based on biopolymers is constrained. The biopolymers poly(limonene carbonate), silk fibroin, chitosan, gelatin, nanocellulose, alginate, polylactic acid, and polyhydroxyalkanoates are ideal for optical applications. The creation of materials with novel functionalities and programmable properties is made possible by the abundance of functional groups present and the potential for manipulating the supramolecular organization of the biopolymers. Here are a few examples of how biopolymers can be used in optical applications.

Displays

Various flat panel display (FPD) technologies, including active matrix liquid crystal displays (AM-LCDs), active matrix organic light emitting displays (AM-OLEDs), and others, are being mass-produced for flexible displays and lighting panels at this time. Organic light emitting diode (OLED) has been adopted as the preferred technology for flexible or conformal displays in the mobile display market due to its inherent advantages of being lightweight, having a high brightness, having a low power consumption, having a broad color gamut, having a quick response time, and having a high degree of flexibility. It is of tremendous practical importance to improve OLED with biocompatibility and human affinity given the demands of

flexible and wearable technology for device biocompatibility and the advancement of bio-photonic research. When electric current travels through a light-emitting diode, it creates monochromatic light, or light with a single wavelength. The semiconductor device produces a P-N junction, with the P-side referred to as the anode and the N-side referred to as the cathode; the region between the two is referred to as the depletion region. Forward bias results in recombination of holes from p-types and electrons from n-types, which results in energy being released as light B. Geffroy et al. (2009).

Being renewable, the enhanced properties of biopolymers are emerging as a promising replacement for inorganic LEDs. Deoxyribose nucleic acid is a remarkable molecule both from the perspective of structure and properties. They are electrically conductive and cheap and are promising materials for use in optical devices. Gupta et al. (2011) and Kobayashi et al. revealed the dipping-coating technology that embeds Ru(bpy)₃²⁺ DNA doping into LEDs as soluble water-soluble DNA polyaniline complexes. OLEDs are traditionally made on rigid glass substrate, and the polymer substrate offers an alternative as flexible material.

Natural biopolymers can be used as substrates for flexible green electronic devices because of their nontoxicity, high mechanical strength, strong biocompatibility, and biodegradability. A translucent paper made of self-assembled chitin nanofibers (CHnF) with strong hydrogen bonds was introduced by Jin et al. The use of low-cost coating is an effective method for LED source conversion, but the lack of reliable and stable white light emitting diodes (WLEDs) in the display industry is one of the significant technological obstacles. Gotta and colleagues used biocomposites of biological (crystalline nano-cellulose and porcine gastric mucus) and organic (luminescent dye) materials to generate white light emission in blue LEDs. Thanks to both the encapsulation properties of proteins (mucin selectively binds to dye molecules through molecular disulfide bonds) and the excellent host matrix of cellulose nanocrystals, the resulting composite material has excellent optical and mechanical properties as well as resistance to heat, humidity, and ultraviolet radiation (self-assembled into nanoscale or layered ordered films may include active ingredients such as nanoparticles). This study lays the path for the development of nonluminescent coatings devoid of rare earth elements for WLED technology.

Additionally, organic thin film transistors (OTFTs), essential parts of electronic displays, are crucial in regulating pixel resolution. Biopolymers have received a lot of attention recently as potential building blocks for flexible TFTs. For instance, the effective preparation of high performance OTFTs was made possible by the use of biocompatible human hair keratin as a supporting substrate and high capacitance dielectric layer. Proton conduction is facilitated by the formation of hydrogen bonds between amino groups and absorbed water molecules in keratin, which has extraordinarily high dipole properties and hygroscopicity. Due to protein denaturing at basic conditions, the constructed devices may also degrade in alkaline solutions. In addition, keratin is a promising carrier material for environmentally friendly, completely printable, and low-cost photo-electric devices due to the abundance of hydrogen bonds and ease of structural alteration in its molecules. As a substrate or gate dielectric for OTFTs or other functional electron devices, cellulose nanofibers (CNF) have also been used.

Solar Panel

Sustainable energy is provided by solar cells, which are also an essential part of energy-conversion machinery and are popular due to their low price and environmental friendliness. Flexible solar cells have recently experienced significant development as a result of their special advantages related to their ability to survive complex manufacturing processes and de-forming. Solar cells are a semiconductor device that provides a steady source of power using sunlight. Due to its low cost and resilience, it has gained wide popularity. Biopolymers such as cellulose, chitosan, pectin, and silk fibroins are increasingly being sought after as optical and electronic devices because they possess lower density and thermal expansion coefficient, higher strength, and elasticity moduli, which reveal tremendous potential for solar cells. Yuqiang and co-workers built flexible organic solar cells using silk fiber fibers that are biocompatible and have silver nanowire grids.

Inverted organic solar cells were used to electrostatically self-assemble chitosan molecules into a highly ordered layer-by-layer (LbL) cathode interlayer film (OSCs). The ordered LbL structure of the chitosan coating decreased the work function of the electrode and enhanced device performance as a result of the formation of interfacial and molecular dipoles. Electronic devices are becoming increasingly interested in biopolymers such as chitosan, pectin, cellulose, and silk fibroin due to their low density, low thermal expansion coefficient, high tensile strength, and elastic modulus. This has demonstrated the immense potential of inexpensive and ecologically friendly solar cells. Moreover, the development of thin protein/peptidyl-based solar cells during the past few decades has paved the path for biocompatible interfaces for wearable electronics.

The silk fibroin (SF) film is a ground-breaking transparent solar cell substrate. The high transmittance of SF film is attributed to a motif consisting of a sequence of amino acids that appears to have weak absorption in the 400–1200 nm spectral range. In order to construct organic solar cells, Liu and colleagues used natural silk fibroin film with silver nanowire as the conductive substrate, achieving superior conductivity, transmittance, and degradation compared to the conventional flexible ITO-PEN substrate. In addition, conductive composites comprised of DNA and bacterial cellulose can serve as the base material, active layer, and bioelectrode for solar cells. Therefore, biopolymers with diverse molecular configurations and topologies offer them with beneficial electric capabilities, robust mechanical qualities, and good transparency, boosting their usage in wearable and smart energy electronics as well as biomedical energy electronics.

Sensors

In the last few years, the development of high-performance, flexible electronics, which are capable of accurately sensing the outside signals, has been a major field of study. Chemical sensors detect chemical substances by translating chemical reactions into electrical signals, playing a major role in food and environmental safety.

Biosensors are sensitive to bioanalytical fields, like diagnostics, therapeutics, and forensics. A high-sensitivity biosensor of alpha-fetoprotein, the tumor-forming protein, was developed by Min et al. The sensor showed favorable selectivity, sensitivity, ultra-low detection limits, high hydrophilic, and good antifouling capability; thus, it was found a high-viability potential in clinical diagnosis. High performance flexible and extensible sensors that can precisely sense external signals have recently emerged as a key study area because of advancements in flexible electronics, materials science, and micro-/nano-processing technology. As an active sensing layer, conductive electrode, and substrate, natural biopolymers and chemically produced polymers with inherent bio-compatibility have been widely used in a range of sensors. In addition to giving the sensors high flexibility, the inclusion of functional biopolymers through doping, surface modification, and chemical group design also enhances the charge transfer in the active sensing layer, raising the sensitivity and detection threshold of the sensors. This section primarily introduces chemical sensors, biosensors, and strain sensors with enhanced performance through molecular modification of biopolymers.

By converting chemical processes into electrical impulses, chemical sensors are sensitive to a variety of substances. This technology is vital for intelligent agriculture, food security, environmental monitoring, and medical diagnosis. Wang et al. developed a functional polysaccharide film-based humidity sensor with high humidity sensitivity by forming a network of hydrogen bonds between water and polysaccharides, which accelerates proton transport in humid environments and exhibits strong responses to humidity. Keratin is an outstanding biopolymer for humidity sensors as an active material for ionic/electrical transduction due to the abundance of functional groups (C = O, C-O, and O-H) on its side chains. These groups enhance the chemisorption of water molecules and facilitate proton transfer in humid environments.

The presence of functional groups such as NH₂, OH, C-N, and C=O on the surface of chitosan film enhances the adsorption of certain active molecules, hence enhancing the sensitivity of sensors. Shantini et al. employed chitosan as the active sensing layer in the creation of a chemical sensor to detect hexanal. For chemical sensing applications, biopolymers with many functional groups that can accurately detect analytes are hence suitable. In the realm of bioanalysis, biosensors have been widely explored for forensic analysis, drug release monitoring, and biomarker identification. By correctly grafting or adding conducting polymers or molecules to biopolymers, which can supplement and improve the performance of biopolymers in biosensors, it may be possible to achieve greater electrochemical activity for the purposes of biological sensing. Wei et al. utilized HA to bind CD44 receptors in order to develop a photoelectrochemical biosensor for the detection of CD44-related cancer with excellent sensitivity. Consequently, the creation of intrinsically biocompatible and electrochemical biopolymers is a potential strategy for producing high-performance biosensors.

Strain sensors, and particularly wearable strain sensors, have attracted a great deal of interest because they may play a significant role in future flexible and wearable electronic systems. In order to perform long-term monitoring of human health and activities, mechanical stretchability has emerged as one of the most essential

characteristics for strain sensors. Rarely these are the vast majority of naturally brittle and chemically unstable biopolymers utilized in flexible strain sensors.

In addition, silk possesses outstanding optical properties, such as high transparency and the capacity to alter sheet crystallinity, highlighting the protein's potential in organic photonics and electronics. Constructing functional biopolymers utilizing simple doping and heating procedures that provide excellent mechanical properties without losing sensing ability is an effective method for producing high-performance, flexible strain sensors.

Diverse chemical and physical alterations equip biopolymers with functional molecular structures that have greater sensitivity, greater optical resolving power, and more flexible features, hence enhancing their eligibility for usage in a variety of functional sensors. It is still challenging to satisfy all practical needs without compromising biocompatibility and sensor performance.

Flexible Electronics

Biodegradable and reproducible biopolymers are in an increasing demand in flexible electronics to replace nonrenewable resources and reduce electronic waste disposal. Several nanomaterials have been combined with biopolymers to offer additional functionality in order to meet the requirements of emerging new technologies that require the combination of mechanical, optical, and electrical capabilities, as well as sustainability and ecologically friendly ways. Natural biopolymers have a variety of exceptional characteristics, including low cost, biocompatibility, superior mechanical properties, and light weight. As a result of these remarkable features, natural functional biopolymers can be developed and enhanced for the production of high-performance flexible electronic devices.

Silks, a family of biopolymer fiber materials, are produced by numerous species, most notably arthropods like spiders, worms, and butterflies. These proteins have a unique nanoarchitecture that provides them with particular properties such as rigidity, strength, and toughness. In comparison to synthetic polymers, they possess superior mechanical properties, biocompatibility, biodegradability, and processability in a variety of macro- to nanoscale shapes and geometries. Silk materials are creating new opportunities in optics, photonics, and flexible electronics due to its transparency, flexibility, and surface patterning simplicity. The ease with which silk can be functionalized with organic and inorganic dopants utilizing either a controlled mesoscopic assembly or a straightforward macroscopic mixing of water-based solutions is one of its primary advantages. Transparent, conductive AgNW/CNF paper for flexible electronics was created using a hot-pressing process. In terms of electrical conductivity, the outcomes were comparable to ITO and sufficient for the development of organic solar cells.

Electronic skins (sometimes referred to as “e-skins”) and flexible strain sensors are fundamental components of flexible electronics and have a wide variety of applications for monitoring human daily activities, HMI, AI, and the prevention and treatment of human diseases. Soft, elastic e-skins may contain an array of

sensors that can mimic the features of human skin and detect ambient conditions. These sensors include of pressure, temperature, humidity, and strain sensors, among others. In an ion gel, Zhao et al. demonstrated cellulose-based electronic skins composed of cellulose, ions, and water (Cel-IL dynamic gel). E-skins exhibited a microstructure resembling brambles with ionic conductivity, high adhesion, and rapid self-healing properties. The biomimetic e-skins were also highly sensitive to certain mechanical strains and human movements, making them a promising tool for monitoring human mobility.

Despite the substantial advances made in recent research on biopolymers and biopolymer-based sensors, integrating the practical application of biopolymers in flexible electronics presents various challenges. While natural biopolymers have been developed as nontoxic, biocompatible, and biodegradable substrates for flexible electronics, other integrated components have limits in this field. To offer biopolymers new extraordinary qualities, such as electronic conductivity, bioactivity, thermostability, 3D conformal properties, and high ion mobility on skins and curved surfaces, more mechanistic research and synthetic methodologies must be created for innovative biopolymers. Finally, it is necessary to develop surface/interface engineering methodologies, substrate fabrication technology, and multifunctional integration of biopolymers-based flexible electronics. Unquestionably, biopolymers offer an exciting opportunity to take advantage of new functional flexible electronics, which are vital to enhancing their application in eco-friendly, durable, and intelligent electronic devices.

Bioimaging

Bioimaging is necessary for comprehending the appearance, structure, metabolism, and functions of cells. Consider sensitivity, specificity, efficacy, optimal clearance, and response to disease biomarkers for the appropriate imaging agent to aid in the diagnosis, evaluation, and treatment of diseases.

Hyaluronic acid (HA) is the only nonsulfated glycosaminoglycan (GAG) that is common in synovial fluid and extracellular matrix (ECM). The range of molecular weights (MW) for HA is vast, from 1000 to 10,000,000 Da. HA degrades into minute molecules as it traverses successively through tissues, lymphatics, lymph nodes, blood, liver, and kidney. Depending on its MW, HA has a number of physiological functions in the body. For example, high MW HA, which is frequently found in loose connective tissues, serves to maintain the water content and cell integrity of the extracellular matrix (ECM), whereas oligosaccharide HA, a degraded form of MW HA, is known to stimulate angiogenic signals in endothelial cells. The concentration of HA in the blood is generally low, with the exception of an abnormal increase caused by altered cell activities such as carcinogenesis, inflammatory response, and immunological reaction. Concerning cancer, HA's biological actions have been extensively researched. Kim et al. reported employing HAQDot conjugates *in vivo* to investigate the effect of chemically modifying HA's distribution within the body. HA-QDot conjugates with a near-infrared emission wavelength and a red emission wavelength were employed for real-time bioimaging of HA derivatives in naked mice. Bioimaging

the temporal evolution of HA derivatives has traditionally required the successive slaughter of multiple animals. Our research provides long-term real-time monitoring of HA-QDot conjugates without the use of naked mice. Regarding their application in tissue engineering and drug delivery, it was described how HA derivatives are dispersed throughout the body according on the degree of HA modification.

Recent research on biopolymers, such as polysaccharides, has centered on the development of novel nanomaterials. Cellulose derivatives, such as carboxymethylcellulose (CMC), are among the promising possibilities for the production of semiconductor nanocrystals. CMC is a pH-sensitive, nontoxic, biocompatible, and ecologically friendly anionic water-soluble polysaccharide synthesized from cellulose with various amounts of carboxymethyl groups (known as the degree of substitution, DS). Consequently, these CMC features provide a vast array of potential for biomedical applications, such as the production of functional capping ligands for illuminating nanoprobe intended for cancer research. Anderson et al. created unique ZnS quantum dots that were stabilized and functionalized by carboxymethylcellulose using an environmentally friendly aqueous colloidal method. In addition, they meticulously characterized the novel ZnS quantum dots. Keeping in mind the colloidal chemistry technique, a thorough analysis and interpretation of the optical properties' dependence on the synthesis parameters (pH of the solution and concentration of precursors) were conducted.

For the regeneration of damaged or infected tissue, biopolymeric in situ hydrogels are crucial. Despite its exceptional biodegradability and biocompatibility in the biological system, only a few number of biopolymeric in situ hydrogels have achieved clinical approval. Hydrogels are networks of hydrophilic polymers that may absorb up to 2000 times their dry weight in water. Hydrogels are formed by hydrogen bonds, hydrophobic forces, and secondary forces such as ionic or molecular entanglements binding polymeric networks. Some hydrogels may have an inhomogeneous physical structure due to clusters of molecular entrapment, ionically connected domains, or hydrophobicity. Due to their injectable matrices, ease of therapeutic agent encapsulation during hydrogel network creation, and little invasiveness into the target location, in situ-generated hydrogels have garnered considerable interest. Chen et al. demonstrated that injectable, in situ-produced hydrogel-based bone scaffolds may be monitored and evaluated nondestructively and noninvasively using ultrasound. In addition, they demonstrated how to produce in situ hydrogels from nanohydroxyapatite, collagen, and chitosan that display good stiffness, low degradation rates, and enhanced blood flow in vivo as determined by ultrasonography. The outstanding features of the bio-polymer-based carbon dots make them excellent for usage in bio-sensing, biological labeling, medical diagnostics, optoelectronic devices, and bioimaging.

Data Storage

Importance is increasing for the creation of functional memory components for information technology, where data storage components are used as building blocks. Data can be stored using electrical bistability, which emerges in devices as two states

with different conductivities at the same applied voltage. Using organic materials for memory devices coupled with solution processing enables large-scale and cost-effective implementation. Several approaches for constructing the active layer of organic memory devices have been proposed. In one arrangement, polymers combined with metal NPs in the polymer-nanoparticle (NP) system exhibit electrical bistability. Given that the optical and electrical properties may be manipulated by varying the composition and size/shape of the nano components, polymer-NPs combinations are attractive as functional materials for a variety of applications. Due to its affinity for metal ions, deoxyribonucleic acid (DNA) is a well-known good template for the production of metal nanoparticles (NPs). Recent research has shown that the processing of DNA for thin-film optoelectronic applications is perfectly compatible with that of conventional polymers, making it an additional interesting optical material.

Hung et al. demonstrated a DNA biopolymer nanocomposite-based photoinduced write-once read-many-times (WORM) organic memory device. The device consists of a single layer of biopolymer sandwiched between electrodes, and electrical bistability is activated by the in situ production of silver nanoparticles embedded in the biopolymer after exposure to light. The device exhibits a switching effect to high conductivity and an excellent retention characteristic above a threshold of 2.6 V. This straightforward technique has the potential to optically alter the properties of DNA nanocomposite thin films, which has promise for optical storage and plasmonic applications. This is accomplished by utilizing DNA's affinity for metals and solution processing.

Optical circuitry, optical data storage, three-dimensional mechanical actuators, birefringent structures, photonic crystals, and bone replacement can all benefit from the fabrication of three-dimensional structures with dimensions ranging from micrometers to nanometers by means of two-photon polymerization. Since the nonlinear nature of multiphoton absorption limits polymerization to the laser's focal volume, structures can be created by scanning a focused laser beam across the resin. Due to optical nonlinearity and the photo-polymerization threshold energy requirement, multiphoton polymerization techniques provide resolutions superior to the laser beam diffraction limit. Correa et al. described building structures with the biopolymer chitosan utilizing two photons and a resolution of around 700 nm.

Conclusion

Today, molecular engineering is one of the most effective methods for resolving inherent limits and enhancing a system's distinctive properties by physical or chemical alterations, hence opening creative paths for the creation of multifunctional bioelectronic devices. In today's culture, electronic devices are ubiquitous, permeating every facet of human activity. Nonetheless, an increasing volume of e-waste is causing environmental difficulties. Therefore, biodegradable electronics has emerged as the most practical and desirable solution to environmental issues, and the biopolymers used in recent applications for functional electronics are examined

in detail in terms of molecular engineering. The notion of bio-based electronics refers to the incorporation of bio-based components into specific electronic equipment, resulting in increased electrical efficiency and a reduction of environmental issues. Several fields, including organic electronics, materials chemistry, and printed electronics, are currently being merged in order to accelerate the development of flexible electronics toward the creation of new, environmentally friendly electronics. Although challenges remain in the biopolymer field for real-world applications, it is highly significant for electronic waste reduction, thus creating novel electronic devices that are environmentally friendly and sustainably designed.

Here, we review the current status of biopolymer design and highlight theoretical and experimental advances in building novel materials. We believe that investigating different parameters effects in biopolymer synthesis and processing offers opportunities for building mechanistic models and exploring the design possibilities of novel materials.

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Mechanical Properties of Biopolymers

10

Samuel Ogbeide Ebhodaghe and Henry Ndibe

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Abstract

There are close similarities between biopolymers and native tissues. The properties of these materials make them suitable for application in regenerative medicine. This chapter includes current research works and reports on the influence of water vapor annealing process, cellulose nanomaterials, pectin materials, cross-linking effects, and others on the mechanical properties of silk fibroin, collagen,

S. O. Ebhodaghe (✉)

Chemical Engineering Department, University of Benin, Benin City, Edo State, Nigeria

e-mail: samuel.ebhodaghe@eng.uniben.edu

H. Ndibe

Department of Chemistry, University of Benin, Benin City, Nigeria

chitosan, gelatin, and alginate biopolymers. While several arguments have been included, the use of these cellulose- and pectin-based materials is undisputed. Recent experiments on mechanical characterization techniques including resilience, hardness, fatigue, torsion, bending, traction, and compression have been highlighted.

Keywords

Biopolymers · Mechanical properties · Regenerative medicine · Characterization

Introduction

The increasing environmental awareness combined with a circular economy policy backed by new rules is pushing plastic manufacturers and consumers to choose environmentally friendly raw materials for their product (Aliotta et al. 2019). State-of-the-art researches based on properties especially in regard to the aspect of environmental sustainability and protection indicate great possible benefits in different industrial applications (Pellicer et al. 2017).

Biomaterials are essential components made from proteins (collagens, gelatin, caseins, albumins), polysaccharides (starch, cellulose, chitin, alginate, hyaluronate), and biodegradable synthetic polymers (polylactic acid, polyglycolic acid) which have been used in several applications due to their advantages, features that include cytocompatibility, high strength and stiffness, recyclable, biodegradable, renewable, and do not cause skin irritation (Horney et al. 2018; Bakshi et al. 2019). However, there are some drawbacks, including moisture absorption, the existence of color, the presence of odor when heated or burned during processing, quality inconsistencies, and limited thermal stability (Oksman et al. 2012).

Interestingly, these problems highlight the need to study the properties of the biopolymers, especially the mechanical properties, making it one of the most important properties needed in industrial application like in packaging industry; a global production capacity of 0.21 million ton was recorded in 2016, 0.5 million ton in 2020, and a further increase in million ton will be expected by the year 2023 (Aeschelmann and Carus 2016–2021).

Recent advances on mechanical properties of biopolymers have focused on the development of biopolymer composite materials for varied applications. For example, (Kaewprachu et al. 2022) synthesized “carboxymethyl cellulose/rice flour composites from palm fruit husk film.” The rice flour could reduce the film strength and flexibility when available at high amounts. Also, Bejagam et al. (2022) examined the different mechanical properties of polyhydroxyalkanoates via molecular dynamics simulations. Synthesis of other composites based on casein, glycerin, enzymes, and synthetic polymers has similarly been reported (Prochon et al. 2022; Zaccone et al. 2022).

Over the years, several researches have focused on the mechanical properties of biopolymer with poly(lactic acid) (PLA) as one of the most commercially successful

bio-based and biodegradable polymers due to its good processability and mechanical properties (Jem and Bowen 2020). Beltra et al. (2007) performed two mechanical recycling processes on PLA to investigate their effects on the degradation, in consideration of the structure and properties during the processes. The first recycling process consists of two steps, namely, accelerated aging and melt processing, while the second recycling process consists of three steps such as accelerated aging, demanding washing process, and melt processing. Instead of a change in the structure and properties, the study revealed that the new introduced process, that is, “demanding washing process,” contributed a negligible decrease on the molecular weight of the PLA. Rather, it was the melt processing that gave rise to the thermo-mechanical degradation. This corresponds to the studies of Hopmann et al. (2015). Therefore, this chapter presents the mechanical properties of biopolymers such as silk fibroin, collagen, chitosan, gelatin, and alginate biopolymers. Recent experiments on mechanical characterization techniques including resilience, hardness, fatigue, torsion, bending, traction, and compression have been highlighted.

Mechanical Properties of Biopolymers

Gelatin

Ge et al. (2017) designed a nanocomposite hydrogel of gelatin reinforced with chitin whiskers. They examined the role of the combination in improving the mechanical properties and gelling ability of gelatin. At different concentrations, the mechanical strength, hardness, fracture stress, and strain were more stable than when tested for pure gelatin. Furthermore, Nuvoli et al. (2020) reported on the structural, thermal, and mechanical properties of fish gelatin reinforced with milled tara gum and glycerol. The gelatin-based films having a high glycerol content could exhibit good thermal stability. However, an excessive increase of the glycerol was also found to have negative impacts on the mechanical properties. In brief, this reinforcement increased the thermal stability and mechanical properties of the composite. Another recent study has also reported the use of “nonequilibrium atmospheric pressure plasma in stabilizing gelatin nanofibers.” In the study, gelatin nanofibers were cross-linked using low-pressure treatment, instead of the usual chemical agents, to address the poor mechanical properties and solubilization associated with gelatin. Figure 1 shows a general overview of the plasma-cross-linked gelatin.

Based on these earlier findings, current researches are investigating the facile approaches and synergistic effects of enhancing the properties. For example, Zheng et al. (2022) immersed cooling-induced gelatin hydrogels into a $\text{Zr}(\text{SO}_4)_2$ dilute solution to resolve the challenge of gelatin poor mechanical properties, which limits the versatile application of the hydrogel. Mechanical tests showed that the properties of elastic modulus, compressive modulus, and strength were about 400, 1192, and 476 KPa, respectively. These values are about 100, 11, and 5 times, respectively, larger than those of pure gelatin.

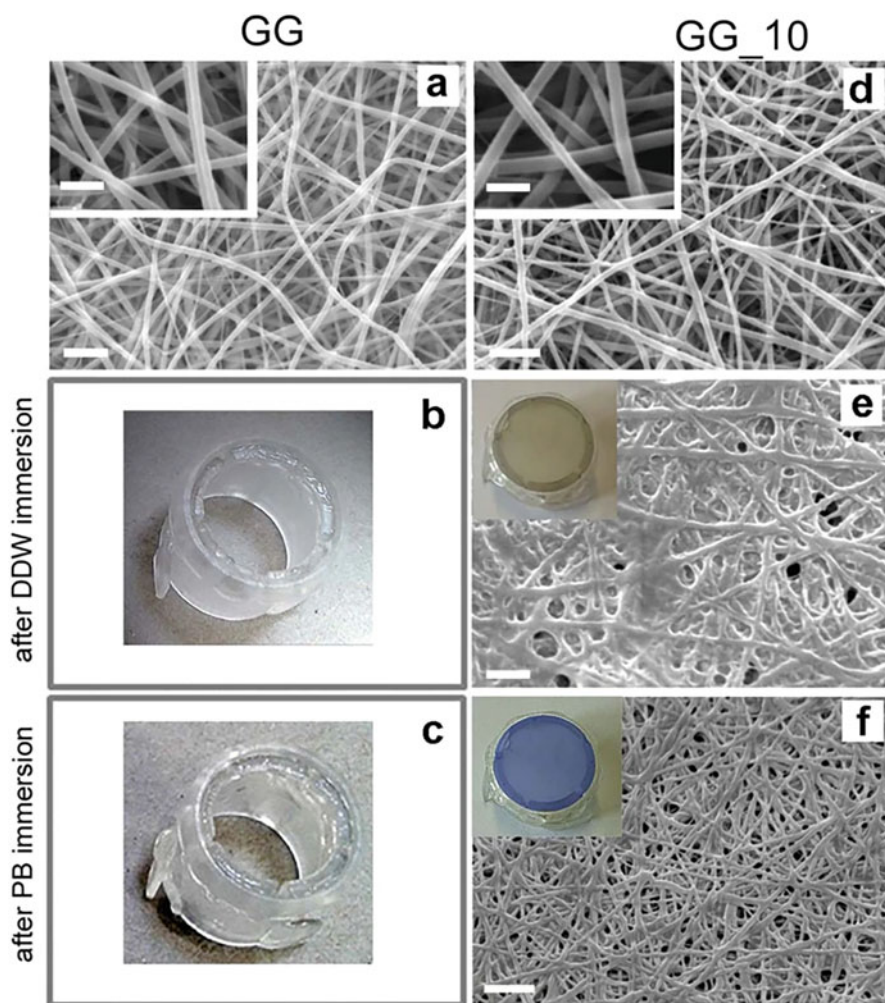


Fig. 1 General overview of plasma-cross-linked gelatin (Liguori et al. 2016)

Current research is also examining the use of other new methods for graphene preparation and mechanical properties, through nano-indentation experiments using atomic force microscopy (Weiss et al. 2018). Similarly, more studies on improving the physical as well as mechanical properties of gelatin after cross-linking with transglutaminase (MTG) are underway (Liu et al. 2020). Its effects on texture properties would also be considered. Helical conformation and coiled conformation of gelatin chains when immersed in water exist in gelatin products, and their ratio influences the mechanical properties of the final material (Rivero et al. 2010). Further, Cai and coworkers (2019) have built on the recent review of (Ebhodaghe 2022). They examined the effect of electrospinning, coaxial electrospinning, and

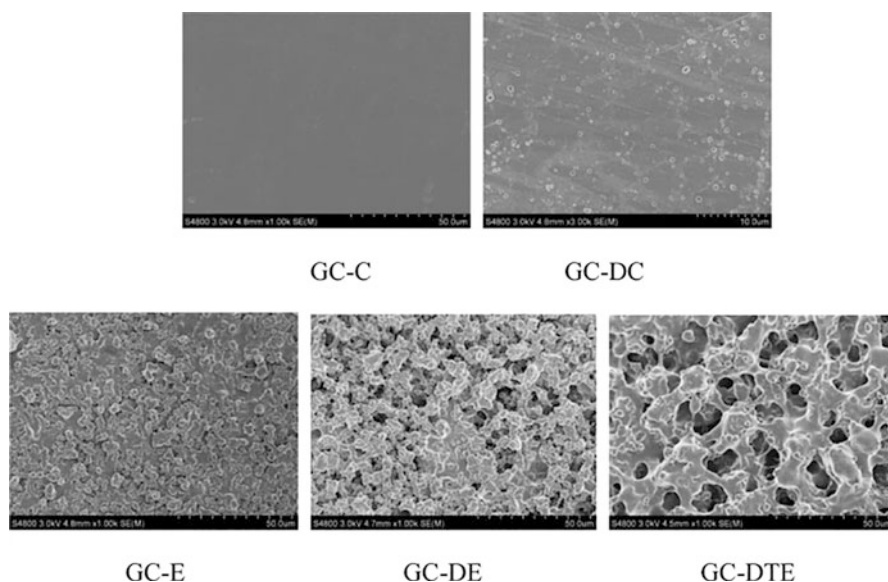


Fig. 2 SEM images of gelatin/chitosan films with and without DAS (Cai et al. 2019)

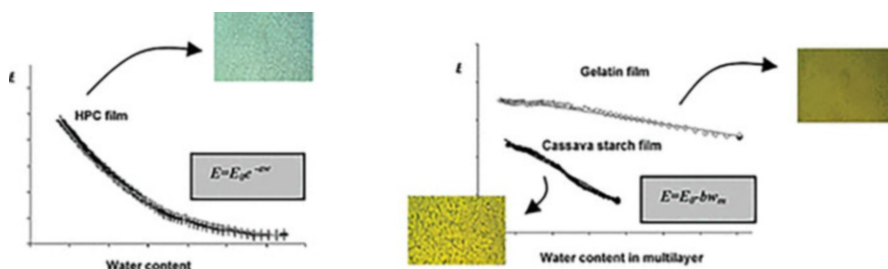


Fig. 3 Influence of water content on polysaccharide-based biopolymer. (Reproduced with permission from Yakimets et al. 2007)

others on the properties and structure of gelatin/chitosan films incorporated with docosahexaenoic acids. Notably, they report that electrospinning and coaxial electrospinning enhanced the mechanical properties and solubilization of the films. Figure 2 shows these in detail.

Effect of water content on biopolymers is shown in Fig. 3.

Silk Fibroin (SF)

Hong et al. (2019) investigated the use of chondrocytes-based silk-GMA hydrogel for the repair of cartilage defect using digital light processing for the 3D printing of the hydrogel. This resulted in good biocompatibility and mechanical strength being

impacted to the hydrogel for sustained tissue regeneration. Similarly, Rajput et al. (2022) prepared photocurable methacrylated SF which resembles the mechanical properties of human tissues. Using the same method as Hong et al. they also report that this scaffold had the same compressive modulus (12 KPa–96 KPa) and degradation (48–91%, in 21 days). This was somewhat similar to those of human tissues. Furthermore, research is ongoing on the possibility of adjusting the mechanical properties of Mo_3Se_3 -PVA-SF nanowire to provide regeneration in tenocytes obtainable from the human bone marrow stem cells. SF and PVA could enhance the mechanical properties of the nanowire, which gives prospects for application in tendon tissue engineering. This is because the molybdenum-based nanowire contributes mechanical strength, reinforcement, high porosity, and biocompatibility to the blend (Subramanian et al. 2022). Moreover, Liu et al. (2022) have examined the synthesis of SF and PLLA electrospun fibers through a hot stretching process to develop textile tissues. While this process enhances the fiber alignment, crystalline nature, and mechanical properties, the SF/PLLA mass ratio reduced their crystallinity and mechanical properties though with increasing degradation rate and surface hydrophilic nature. Overall, the properties of physical, biological, and mechanical strength were enhanced for advanced nanotextile tissue construction. Taking a different approach, Cho et al. (2022) investigated the influence of water vapor-annealed SF on the indentation modulus and hardness of graphene oxide-SF through nano-indentation experiments and finite element analysis (FEA). Water vapor annealing process can be used to enhance the indentation modulus and hardness of the bionanofilms because of close similarity of the FEA and the nano-indentation. Another recent development is the use of cellulose nanocrystals (CNC) in enhancing the mechanical properties of SF (Poggi et al. 2022). Result of the study on mechanical response and yielding transition of composite gels by Poggi et al. showed that gelation occurs rapidly and viscoelastic response was more in the presence of CNC. Yield strain of the gels reduced at higher shear rates probably due to the shear-induced compaction.

Collagen (COL)

The normal human body consists of one-third COL, even as it provides structural and mechanical stability. This is why COL films usually find applications in tissue engineering and regenerative medicine (Ebhodaghe 2021, 2022). Studies have shown that collagenous materials show different mechanical properties in different conditions. The effect of cross-linking on tensile properties of COL is shown in Table 1. More so, the fact that the same COL materials could display different mechanical properties influencing their usability (while depending upon their states) makes case for a closer study.

The effects of cellulose-based nanofibers have been intensely investigated for different biopolymers, as shown in Fig. 2. On COL, the compression strength and fracture strain were improved upon when the nanofibers were impregnated on COL (Lohrasbi et al. 2020).

Table 1 Effect of cross-linking on tensile strength of collagen biopolymer

Cross-linking treatment	Nature of collagen	Control (MPa)	Time (h)	Amount (%)	Tensile strength (MPa)	References
Proanthocyanidin PBS solution	Demineralized	10.8 ± 4.9	4	0.5	17.7 ± 9.4	Elango et al. (2016)
EDC in 70% ethanol	Demineralized	–	24	–	408 ± 39.5	Sheu et al. (2001)
Glutaraldehyde	Dentinum reinforced	37.7 ± 4.5	0.083	1	37.3 ± 5.6	Leung et al. (2012)
Glutaraldehyde	Electrospun	43.70	24	5	44.13	Gillette et al. (2010)
Sorbitol	Fish collagen	2.3 ± 0.4	–	0.05	4.8 ± 0.8	Gobeaux et al. (2008)
Glycerol	Fish collagen	2.3 ± 0.4	–	0.05	4.1 ± 0.7	

Bose et al. (2020a) examined the properties and performance of COL films in both dry and wet conditions under different loading and environmental conditions (in air and in water). In water, the samples show an increased stiffness condition, while reduction in modulus was reported in dry condition. Also, the Tan values were 0.05–0.75 for dry samples and 0.12–0.24 for hydrated ones. Further, they investigated the fracture behavior of COL in water and in dry conditions. The utilized specimens of COL films (i.e., a single-edged notched tension) displayed reduced tensile strength and fracture energy, while failure strain was significantly increased in wet conditions (Bose et al. 2020b). Building on these findings, Ma et al. (2022) studied the effect of calcium spraying on the mechanical properties of COL. The samples were sprayed with Ca before and after drying. When sprayed with Ca before drying, the samples displayed improved elasticity and stiffness, while spraying after drying only improved toughness of the samples. These were because Ca penetrated the film in the former, while it only generated a protective covering of Ca/alginate on the membrane surface of the latter. Also, the study shows that while the samples developed a protective layer, the COL retained their integrity for the latter.

Taking a different approach, Andonegi et al. (2022) examined the role of ionic liquids and wool on the mechanical properties of COL, for possible varied applications. The composites were obtained by compression molding, and this made the COL films homogeneous. This presence of ionic liquids in the composites enhanced the mechanical properties of COL, because of the obvious increase in tensile strength.

Chitosan (CS)

Presently, studies are exploring several techniques of enhancing the mechanical properties of CS. One of such is the use of atomic force microscopy (AFM). AFM is a microscopic technique that can be used to investigate a wider range of samples than the scanning electron microscopy (SEM). This is because it can be used in air or liquid unlike the SEM, which is usually adopted in vacuum. The usefulness of the former in tissue engineering lies in the ability to measure more physical parameters than pure topography (Joshi et al. 2022). As such, Luna et al. (2022) examined the influence of temperature and pH on the mechanical properties of CS films. PeakForce-QNM AFM was used for this, with CS being at nanoscale. Minute changes in pH and temperature had effects on the structure and mechanics of the film, though this was not the cause of changes in the COL film surface free energy. Also, another study (Gotla et al. 2022) combined CS hydrogel at different pH values and anionic sodium dodecyl sulfate (SDS) which was added. These ionic surfactants hold potential to improve the mechanical strength of CS. At acidic pH conditions, CS possesses superior mechanical strength unlike when the conditions are basic. The study showed that CS composite hydrogels are similar to stretched rubber and can undergo load-dependent crystallization at basic pH conditions. This enhancement can inspire new mechanical applications based on the combinations. Also, the role of

pectin on mechanical properties of CS was observed when Akalin et al. (2022) synthesized CS-based and CS-/pectin-based silver nanoparticle films and confirmed that the presence of pectin improved the mechanical properties of CS.

Alginate

Alginate is an ubiquitous polysaccharide present in brown seaweed. As a common viscosifier and gelling agent, it can be used in different industries, ranging from food to pharmaceutical and biotechnology.

Considering the effect of gel network on mechanical properties of alginate, Schuster et al. (2017) examined the relationship between network structure and functional properties of capillary alginate gels for muscle fiber formation. They found a correlation existing between the nanostructure of gels and mechanical properties of the biopolymer. Moving forward, Notermans et al. (2019) also examined the relationship between the mechano-biological adaptation of rat Achilles tendons and changes in their constitutive mechanical properties and biomechanical behavior. They compared creep and stress relaxation to experimental data to generate a generalized computational model for the Achilles tendon. Also similarly, the effect of high sugar content on microstructure mechanics and release of calcium-alginate gels have been reported (Lopez-Sanchez et al. 2018). Sugar contents greater than 15 wt% reduced the extensibility of alginate molecules. They showed the structure-function relationship of the gels. The release of sugar from calcium alginate gels having sugars of 15 wt% and 30 wt% was similar to Fickian diffusion mechanism and others. Recently, Matthew et al. (2022) developed an alginate-hyperbranched polymer complex (HBP) from its addition to alginate structure. A vortex fluidic device was used to control the physicochemical, structural, and mechanical properties of the hydrogel. Incorporating the HBP complex to the hydrogel structure increased their swelling, diffusion coefficient, and failure mechanical properties though the bulk structure was the same. This was similar to the rate of degradation which also was largely unaffected. Overall, the HBP changed the structural and mechanical properties of alginate, while the degradation property was modified.

Mechanical Characterization Techniques

These refer to recent experiments conducted on resilience, hardness, fatigue, torsion, bending, traction, and compression of materials. Some of these techniques are shown in Fig. 5.

Resilience

Recent studies have reported experiments on the impact of amylases on biopolymer dynamics during storage of straight-dough wheat bread (Bosmans et al. 2013) and

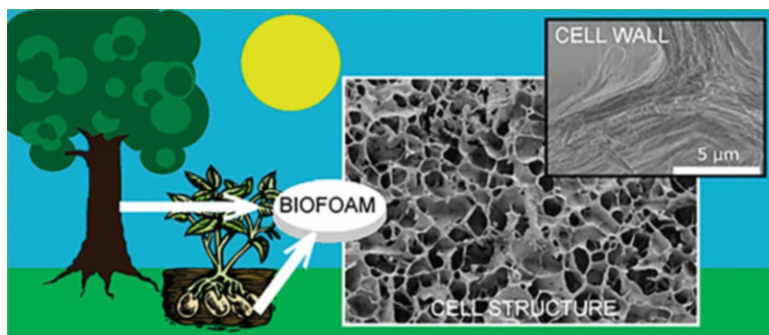


Fig. 4 Cellulose nanocomposite-based biopolymer source. (Reproduced with permission from Anna et al. 2011)

the shear modulus and fracture properties of biopolymer double networks (Lwin et al. 2022). Results from this latter experimentation showed that mechanical properties could be changed from being compliant and ductile to stiffness by a secondary network of flexible fibers, which occur only when a primary network of stiff fibers is rigid. This observation is responsible for the resilience and tenability of biopolymer double networks.

On the optical and mechanical properties of self-repairing pectin biopolymers, the influence of water on pectin repair has been reported (Pierce et al. 2022). This water-induced self-repair of pectin was enhanced by its physicochemical properties which are related to some reparative and regenerative processes.

Hardness

Factors that could influence the hardness of biopolymers obtained from potato and plantain peels have been examined (Castro et al. 2021), as shown in Fig. 4. The recent study related the combination of urea, glycerine, acetic acid, and water. Also, reports on the maximum hardness of albumin, COL, and CS with single-walled carbon nanotube (CNT) concentrations of 0.01 wt%, 482 ± 10 , 425 ± 10 , and 407 ± 15 MPa, respectively, have been presented (Gerasimenko et al. 2021).

Torsion

A recent study designed a mechanical system to determine the torsion modulus of polymers. They further subjected polytetrafluoroethylene to torsional stresses. This was important because of the possibility to design polymer products and determine if the strength needed to prevent fatigue was present in the polymers (Piedade et al. 2020).

Bending

It is possible to enhance the bending mechanical properties of hydroxyapatite (HA)/PLCL sandwich beam scaffold for use in tissue engineering applications. The presence of the beam was needed to enhance the critical stress and the fracture absorbed energy. Also, the bending mechanical properties were improved by sandwiching the HA/PCL scaffold with the PLCL layers (Hazwani and Todo [2022](#)).

Furthermore, Hazwani and Todo ([2021](#)) designed composite HA/PLLA and HA/PLCL via template and freeze-drying methods. Here, the three-point bending was used to determine the bending mechanical and fracture properties of the porous composite beams. The presence of PLLA and PLCL enhanced the flexural mechanical properties of the HA. Specifically, PLLA caused a higher modulus and fracture strength while PLCL a higher fracture energy. By extension, (Alvarez-Blanco et al. [2022](#)) recently examined the failure of additive manufacturing components when subjected to three-point bending tests of strength and crack morphology. In other words, they analyzed the fracture morphology of 3D printed lattice core PLA samples. In brief, process parameters can influence the mechanical behavior and failure of the components made from additive manufacturing.

Traction

Researches have estimated the effect of cellular traction forces on the morphogenesis of capillaries via advanced modeling of fibrous biopolymers (Ah-Hing [2022](#)). The study highlights independence among density, displacements, and tractions.

Compression

Chen et al. ([2022](#)) has considered the combination of biopolymers and fiber inclusions as an alternative to reinforcing soft soil. They presented the synergistic effect on failure pattern, strength enhancement, stress-strain characteristics, and the reinforcement mechanism of soft soil. Overall, the biopolymer enhanced particle bonding force and reinforcement efficiency of soil fibers, while fibers reduced soil brittleness with residual resistance after post-peak failure.

Fatigue

Ni et al. ([2020](#)) investigated the performance of soils enhanced with economically adopted natural polymers in unconfined compression strength tests and fatigue loading tests (Peron et al. [2018](#)). This is because biopolymers can affect ground improvement depending on their types. The study argues that as a biopolymer soil strengthener, xanthan gum can enhance the stabilization and fatigue life of soils even

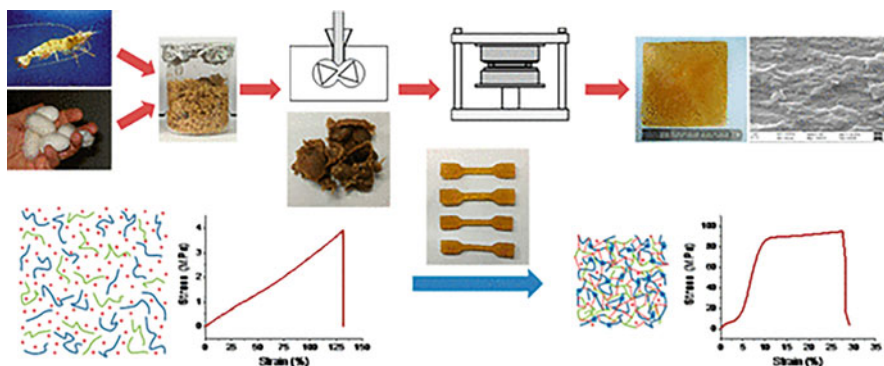


Fig. 5 Mechanical characterization techniques. (Reproduced with permission from Meng et al. 2019)

when they are subjected to continuous loading. Also, Luo et al. (2020) performed fatigue-to-failure experiments for fatigue life assessment of filled rubber by hysteresis-induced self-heating temperature. It became possible to examine the fatigue life of the rubber under the condition of steady-state temperature increase. This is due to a possible relationship between temperature increase and fatigue life. The fatigue parameter used was the maximum principal strain, and this was important in deciding the S-N curve (Fig. 5).

Conclusion

Recent studies on enhancing the physical and mechanical properties of biopolymers have been presented. Notably, pectin- and cellulose-based nanocrystals are materials that can be applied to improve the mechanical properties of several biopolymers. Also, well-organized hierarchical structures have no real effect on the interfacial combinations of molecules. While water vapor annealing process could be widely employed for several biopolymers with poor mechanical properties, more research is needed to investigate how the annealing process could directly affect the interfacial properties between graphene oxide and silk fibroin layers. Challenges of processing biopolymers include intrinsic limitations in experimental methods (Jung et al. 2015), cost-competitiveness, and polymer choice limitations to density and temperature-based properties (Van de Velde and Kiekens 2022).

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Thermal Properties of Biopolymers

11

Amrutha S. R., Rejimon P. K., Suja N. R., and Alson Mart

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Abstract

In the context of sustainable development, the use of polymers of natural origin has gained a lot of significance. Degradability, nontoxicity, a wealth of natural resources available as raw materials, and renewable nature are the main characteristics that are prioritized when employing biopolymers. These polymers have a wide range of applications, including medication administration, tissue engineering, tumor ablation, biomedicine, and food packaging. Their weak thermal and mechanical stability, however, is a drawback. The four most studied biopolymers, cellulose, starch, chitosan, and poly(lactic acid), have all been the subject of thermal investigations, which have been attempted to be analyzed in this chapter.

A. S. R. (✉) · R. P. K. · S. N. R. · A. Mart

Post Graduate and Research Department of Chemistry, Maharaja's College, Ernakulam, India

e-mail: amruthasrajan@maharajas.ac.in

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The thermogravimetric analysis and differential scanning calorimetric methods gave an idea on the thermal stability, degradation steps, and glass transition temperature and crystallinity of the samples. Various studies on effect of different parameter affecting the thermal degradation are also highlighted. Studies have proved that the use of other polymers, crosslinking agents, and nanofillers blended along with biopolymer has improved the thermal stability. These studies would provide an insight into further development of methodologies to improve upon the properties of natural polymers.

Keywords

Thermal Properties · Cellulose · starch · Chitosan · Poly (Lactic acid)

Introduction

Biopolymers have recently gained popularity in industry because of their compatibility, renewable nature, nontoxicity, biodegradability, sustainability, and environmental friendliness. More and more polymeric biomaterials from synthetic and natural sources are being developed as a result of many applications in tissue engineering and restoration, as well as specialized fields like drug supply, nanotechnology, and therapeutical applications. They are extensively utilized in farm products, biomedical gadgets, dental implants, food additives, smart food containers, and other hygiene products on the commercial market. Biopolymers are mostly used in implantations, particularly those with electrical components and those whose temperature-dependent features are crucial for interacting with biological tissues. The majority of biopolymers used in implants has electrical components and/or possess temperature-dependent properties that are essential for interfacing with biological tissues (Ebnesajjad 2013).

Before being used, plant and animal resources must be modified to have qualities that are acceptable for a number of reasons. High molecular weight polymers usually require additives like antioxidants, plasticizers, lubricants, mold release agents, reinforcing agents, and UV stabilizers in order to alter the properties of the polymers and produce novel compounds. A material's thermal behavior is a key factor in defining its characteristics because it is closely related to morphology and has an impact on its therapeutic efficacy (Yaashikaa et al. 2022). Thermal analysis should be performed across a wide temperature range so as to assess various process parameters such as thermal degradation rate, stability, polymer shrinkage, glass transition temperature (T_g), melting temperature (T_m), reaction crystallization temperature, amount of crystallinity, effect of sterilization methods, expansion, insulation, and storage. Mechanical characteristics and thermal behavior are related because below T_g , a polymer is glassy, and above T_g , a polymer is rubbery and a polymer with a regular chain structure can be recognized with a characteristic melting temperature (T_m).

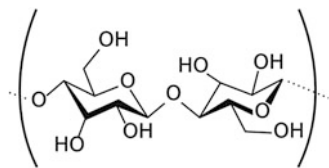
The naturally derived polymers are classified based on a variety of variables, including biodegradability (biodegradable and nonbiodegradable biopolymers), origin (made from natural sources and fossil fuels), thermal condition response (elastomers, thermoplastics, and thermosets), composition (blends, laminates, and composites), and raw material source (natural, synthetic, and microbiological biopolymers) (Van de Velde and Kiekens 2002).

This chapter provides an insight into the different studies carried out in analyzing the thermal behavior and thermal stability of the most relevant and significant biopolymers that had been researched the most. Four biopolymers, namely, Cellulose, Starch, Chitosan, and Polylactic acid, were chosen from among the various biodegradable polymers available. Due to their low thermal stability, these polymers cannot be applied to a wide variety of biological applications, despite their widespread use in such fields. Modern calorimetric techniques, such as differential scanning calorimetry (DSC), temperature-modulated differential scanning calorimetry (TMDSC), adiabatic calorimetry (AC), and fast scanning chip calorimetry (FSC), are employed for proper quantitative thermal analysis of the metastable, semicrystalline, and amorphous biopolymers. Thermal analyses enlighten many transitions in the polymeric matrices and among them, melting, crystal rearrangements, decomposition, enthalpy relaxation, glass transition, crystallization are only a few.

Cellulose

An important factor contributing to global warming is rising carbon emissions. The use of biodegradable and eco-friendly materials initiates substantial change in global climate. The need for composite manufacture has been steadily rising in a number of industries, which has led to an issue with the accumulation of a vast quantity of wasted composites that also requires safe disposal. Therefore, reducing these issues, without compromising on the advantages is a challenging area of research. Due to important benefits including biodegradability, ease of handling, low energy consumption during processing, abundant availability, and high-specific strength, fibers made from natural resources are widely employed in the manufacture of composite materials (Almedia et al. 2006). A linear homopolysaccharide with -D-glucopyranose units connected by -1-4-glycosidic linkages makes up the main component of cellulose (Fig. 1).

Fig. 1 Structure of cellulose



The mechanical properties of naturally available fibers depend on the chemical constituents, growing environment, the lifetime of the species, and retting techniques (Athijayamani et al. 2015). According to studies, mixing cellulose with other polysaccharides like pectin and chitosan may increase flexibility, steadiness, and transparency (Yaradoddi et al. 2020). Additionally, cellulose has poor interfacial adhesion, thermal instability, and water sensitivity, which reduces the appeal of cellulose-based composites. Such technical challenges can be overcome by suitable functionalization and prior treatments of cellulose (Polman et al. 2021). The resources for cellulose are plentiful in many nations, and cellulosic material made from agricultural production can be used to create pure cellulose. Comparing the properties of pure cellulose with corresponding composites showed that the thermal and mechanical properties become promising in the latter. Due to the nature of the materials used to make composites, the production of nanocomposites enhances the qualities. In terms of the applications for these materials, the thermal characteristics of composites are crucial.

Thermal Properties of Cellulose

Various researchers observed that heating dry cellulose from 120 to 470 K using DSC does not exhibit a first-order phase transition. As a result, only flat sample baselines are possible to acquire in DSC curves. A significant endothermic peak caused by water vaporization can be seen in the temperature range of 273 to 400 K when cellulose that has absorbed a little amount of water is examined by DSC. The amount of water determines the peak temperature of vaporization. Any excess moisture in dried cellulose can be assessed by taking the endothermic peak of vaporization as a suitable index. Natural cellulose is a crystalline polymer with a 20–90% crystallinity range. Natural cellulose's crystallinity varies depending on the type of plant it comes from; for instance, kapok's crystallinity is only about 30%, whereas jute's is over 70%. In most cases, solid-state NMR, infrared spectroscopy, and X-ray diffractometry are used to determine crystallinity.

Thermal Properties of Cellulose Composites

Thermal stability is an important element when evaluating the possible applications of composites in several industries (Pyda 2001). Researchers were able to construct composites with improved design and processing parameters and create high-performance polymers with improved thermal stability by studying degradation behavior (Pyda 2002). The two main processes in the thermal deterioration of polymeric composites are the removal of volatile matter and the depolymerization process (Cheremisinoff and Cheremisinoff 1996). Low-molecular-weight molecules, like water, evaporate as the initial stage of thermal breakdown. A reduction in thermal stability could be the result of the hydrolysis of the polymer due to the water absorbed by hydrophilic cellulose (Yano et al. 1976). The highest weight loss

occurs during the second degradation stage, which is also the most important indicator of the heat stability of polymer composites. Researchers were able to create composites with the best possible design and processing circumstances by studying degradation behavior. They were also able to create high-performance polymers with improved thermal stability (Li et al. 2014).

Thermal Properties of Nano Cellulose Composites

While thinking about prospective applications, it is also crucial to understand the thermal behavior of nanocellulose and its thermoplastic composites during mixing and extrusion. Nanocellulose degrades at a temperature between 200 °C and 300 °C; thus, during the manufacturing process, the compounding temperature must be kept under control at 200 °C to avoid the degradation of nanocellulose (Nepomuceno et al. 2017). The thermal stability of the nanocomposites may highly varied by the source of the nanocellulose, the kind of matrix, the processing methods, and the drying method. Thermal stability of nanocomposites is affected by incorporation of nanocellulose. There are often two main steps to the thermal degradation of polymeric composites: the removal of volatile substances or the evaporation of water and the depolymerization process, respectively (Lizundia et al. 2015). Low-molecular-weight molecules, like water vapor, are typically vaporized as part of the first stage of heat degradation. A decrease in thermal stability could result from the hydrolysis of polymers caused by the water absorbed by hydrophilic nanocellulose (Srithep et al. 2013). The highest weight loss occurs in the second degradation stage, which is also the most important indicator of the heat stability of polymer composites. Kilzer and Broido postulated two distinct mechanisms for cellulose degradation: cellulose dehydration, an endothermic process that results in “dehydrocellulose,” and cellulose depolymerization, which competes with evaporation or dehydration. The first mechanism produces dehydrocellulose which will break down into gases and char residue (Kilzer and Broido 1965). The intermolecular interaction between the nanocellulose and the polymer matrix has a significant impact on the thermal stability of composite materials. Strong intermolecular bonds may increase the bond dissociation energy required for macromolecule chain cleavage (Maiti et al. 2013). Therefore, high degree of dispersion of nanocellulose and significant interfacial adhesion between polymer and nanocellulose are necessary for enhancing the thermal stability. The addition of nanocellulose enhanced the thermal properties of the composites, with increases in its crystalline phase and T_m being especially noticeable. As a result, the thermal characteristics of nanocellulose composites may be improved. The integration of nanocellulose as a nucleating agent may boost the overall crystallinity and crystallization rate of nanocomposites. The thermal characteristics of nanocomposites were, however, impacted by the poor dispersion and clustering of nanocellulose, particularly at high concentrations. The distribution of nanocellulose and the interaction of nanocellulose with the polymer matrix can be improved through layer-by-layer deposition, which eliminates agglomeration and considerably enhances the characteristics of the composites.

Cellulosic nanofibers were investigated using TGA as a reinforcing phase in PLA/CNF composites. Investigated was the thermal degrading behavior of the membrane with and without the inclusion of cellulose nanofibers (Wang et al. 2020). The initial breakdown temperature of every PLA/CNF composite was greater than that of PLA, which suggests that the addition of cellulose nanofibers improved the composites' thermal stability. The derivative thermogram (DTG) analysis shows that cellulose nanofibers have two temperature peaks, one at about 40 °C, which is associated with the hydrophilic groups of the nanofibers, and the other at about 346 °C, which was related to the dehydration processes and volatile product generation through chain scission and degradation. There is only one decomposition peak for PLA at 331 °C. Moreover, the initial degradation temperature and maximum degradation temperature of PLA/CNF composites were increased by 20 °C and 10 °C after the addition of PLA/Cellulose composites, 5.0 wt% of CNF, respectively. It can be concluded that the PLA/CNF composites have an improved thermal property compared to the PLA.

TGA measurements were used to assess the thermal stability of cellulose, PVA, and its nanocomposites. Similar patterns may be seen in the TGA thermographs of PVA and their nanocomposites, specifically three primary regions of weight loss. Due to water evaporation, the initial zone (75–160 °C) shows about 5–10% weight loss. The structural degradation of PVA nanocomposite films causes the second transition region (260–390 °C), which has a total weight loss of about 74%. The third region, which was around 390 °C, was caused by the dissolution of carbonaceous materials or the cleavage of backbone of bio composite films. At 500 °C, the overall weight loss in this range exceeded 95%. This demonstrates that cellulose and nanocellulose fiber can increase the thermal stability of PVA, proving that the PVA matrix's increased thermal stability is caused by a strong hydrogen bond between the hydroxyl groups of nanocellulose (Sultana et al. 2020).

The poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) matrix was used to combine the rigid rod-shaped CNC with flexible CNF to create the nanocomposites. The T_c and T_m of PHBV composites increased as a result of the addition of CNC and CNF. A decrease in the X_c results from the addition of cellulose nanoparticle, which stimulated the nucleation of PHBV matrix crystallization. However, compared to the CNC/PHBV composites, the CNF/PHBV composite displayed an enhanced crystallinity (Jun et al. 2017).

Thermal analysis of NCC/gelatin bio-nanocomposites revealed that the addition of NCC raised the degradation temperature by 7–9 °C, enhancing the thermal stability. The thermal stability of CNF/PVOH nanocomposites was reported to have significantly increased. The thermal breakdown temperature of composites rose from 273 °C to 340 °C in comparison to pure PVOH with the incorporation of 10% CNF. Generally, introducing nanocellulose to hydrophilic composites improved their thermal properties (Ben Cheikh et al. 2018).

Studies using TGA and DTG for PVA/NCF composites and pure PVA show a three stage process. These occur between 50 °C and 181.7 °C, between 181.7 °C and 342.7 °C, and between 342.7 °C and 500 °C. The PVA/NCF composite experienced

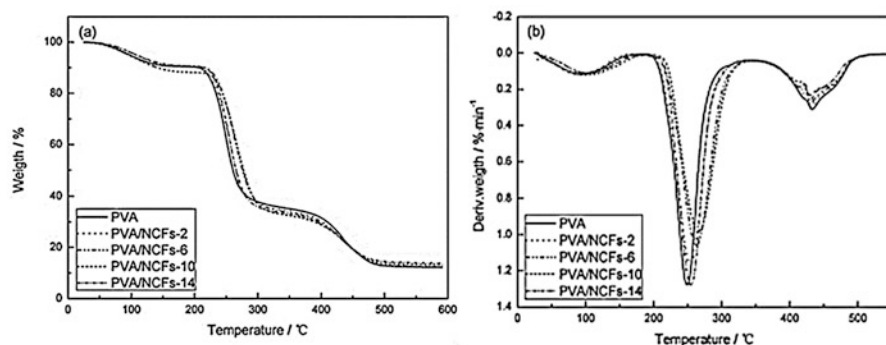


Fig. 2 TGA (a) and DTG (b) curves for the PVA and PVA/NCF composite. (Adapted from reference Li et al. 2014)

its first degrading phase at a temperature that was a bit higher than that of pure PVA (Fig. 2).

The second degradation process in PVA/NCF occurred at a temperature that was higher than that of pure PVA, indicating greater thermal stability for PVA/NCF. With the addition of NCFs to PVA, the maximum decomposition temperatures for the first step increased by 0.4 to 9.1 °C and those for the second process increased by 4.9 to 16.9 °C. This means that a significant increase in the thermal stability of PVA/NCF was observed by the addition of small quantities of NCF (Li et al. 2014).

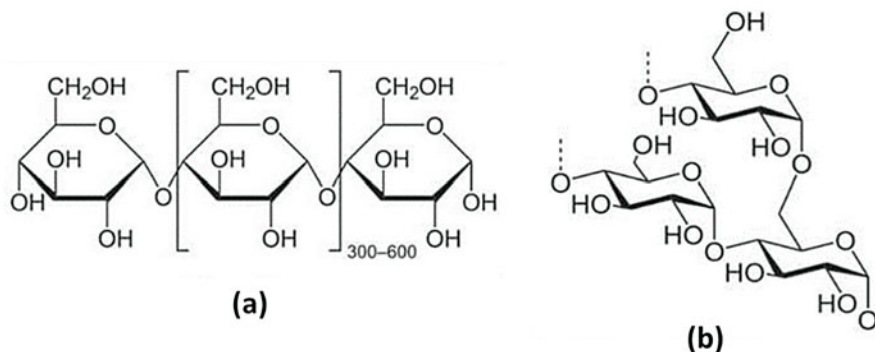
Starch

Starch is a biodegradable semicrystalline polymer made up of 1, 4- α -D glucopyranosyl fragments present in amylose and amylopectin moieties. Both amylose and amylopectin are formed by chain polymerization techniques of D-glucose repeating units, but the only difference is that the first one is a straight chain polymer and the latter is branched. The amorphous character of starch is caused by amylose, whereas the crystalline nature is caused by amylopectin. The content of amylose and amylopectin varies in starch obtained from various sources (Table 1) (Fig. 3).

Starch-based polymers are promising and drew attention because of their easy availability, low cost, and biodegradability. However, native starch owns poor mechanical and thermal stabilities, excessive hydrophilicity, and brittleness. The properties of starch could be improvised by making modifications such as derivatization of the hydroxyl groups or making composites with other additives. To increase the flexibility and decrease the brittleness, plasticizers are added to starch (Vieria et al. 2011). Common plasticizers for the starch biopolymers are polyols (glycerol, sorbitol, polyethylene glycol etc.), formamide, urea, and citric acid. Starch-based thermoplastics have applications in food packaging or in agriculture (Molavi et al. 2015).

Table 1 Amylose-amylopectin composition of starch from different sources

Source	Amylose (in %)	Amylopectin (in %)	Crystallinity
Rice	20–30	70–80	38
Potato	23–31	69–77	23–53
Cassava	16–25	75–84	31–59
Wheat	30	70	36–39
Corn	28	72	43–48

**Fig. 3** Structure of (a) amylose and (b) amylopectin

Thermal Properties of Starch and Its Esters

When starch is heated in water to 60–70 °C, it loses its crystalline nature and becomes a semisolid and the process is called gelatinization (Donowan 1979). In general, the thermal properties of polymers consist of the determination of melting point, decomposition temperature, glass transition temperature, thermal expansion coefficient, modulus of elasticity, thermal conductivity, specific heat, and density. There is a correlation between the amylopectin/amylose ratios with the thermal stability (Liu et al. 2013). Thermal decomposition temperature and the sequence of activation energy increase with amylopectin/amylose ratio.

Glass transition temperature T_g is an essential parameter used for identification of plastics. The transformation from hard to soft in amorphous polymers occurs at this temperature. Shorgen, R. L. has prepared starch acetates with varying degrees of substitution (Shorgen 1996). Derivatization of starch hydroxyl groups helps to provide water repellent nature to the polymeric film. The acetylation of amylose-rich starch gave a more flexible film compared to the fully acetylated normal starch. This is because of the reduction of the crystalline structure of amylose after derivatization. He arrived at the conclusion on basis of his observations that the T_g decreases with increase in the degrees of substitution for dry starch acetate. In addition the T_g decreases with the increase in moisture content.

Rudnik et al. carried out thermal analysis of starch succinates and obtained a result that indicates the thermal stability of starch succinates increases with increase

in the substitution (Rudnik et al. 2005). Stabilities of succinates of starch obtained from different sources are comparable. Similar results have been reported in the literature (Thiebaud et al. 1997). Thermal stability of esters of starch is attributed to the lower number of free starch hydroxyl groups since the decomposition mechanism involves the dehydration between the hydroxyl groups of starch.

Thermal Properties of Starch Composites

The thermal decomposition of starch-based polymers is one of the main difficulties we encounter while employing them. Thermogravimetric analysis (TGA) is a method for measuring the heat stability of polymers that is often employed. A fine controlling over the heating rate, atmosphere, and mass of the substance gives a better analytical result. Thermal experiments are done in excess water condition as well as in water free condition in order to simulate their uses in aqueous and dry processes.

The cautious application of additives can adjust the thermal characteristics of starch-based polymers. The fabrication of nanocomposites of starch with various clays such as montmorillonite, hectorite, and kaolinite improves the thermal degradation temperature (Banna et al. 2020). Incorporation of TiO₂ nanoparticle along with starch causes advancement in the thermal stability.

The inclusion of Aloe Vera gel with starch-based composite films has made significant enhancement in the antimicrobial nature of the film. Since the components of Aloe Vera have antimicrobial activities, it is an efficient substitute for other inorganic antimicrobial agents (Bastarrachea et al. 2011). Hence, it increases the resistance and shelf life of the film, as it is important when it is used as a packing material. The thermal stability of a film of starch-chitosan was achieved by Bajer et al. and made a comparison with the influence of inclusion of Aloe Vera gel in the film (Bajer et al. 2020). The maximum degradation temperatures obtained for starch film, starch-chitosan film, and AV gel-incorporated starch-chitosan film were 293 °C, 298 °C, and 302 °C, respectively. The alteration of the thermal resistance of starch-based films by the inclusion of AV gel is attributed to several reasons like their aggregation, intercalation, and active impact within the starch medium. The thermal stability of pure starch with varying concentrations of AV gel was evaluated by Gutiérrez and Alvarez and found that the weight loss during heating is less with the one with higher composition of AV gel (Gutiérrez and Alvarez 2016). The impact of curcumin-loaded nanoparticles and AV gel on banana starch was examined by Nieto-Suaza et al., and they were able to derivatize banana starch. In all cases, they observed three stages of decomposition, first stage at 105 °C, due to the loss of water molecules. Second stage observed in a range of 180 °C and 250 °C and the third stage is above 250 °C, which is attributed to the complex degradation process in the structure of starch.

For enhanced efficiency, starch-based polymers are compounded with synthetic polymers including polylactic acid (PLA), polyvinyl alcohol (PVA), and polyester. Such plastics are made soft by the inclusion of plasticizers. Among various

plasticizers, ascorbic acid has antioxidant properties and is found in various vegetables and fruits. Hence, ascorbic acid-blended films find applications in packaging, edible films, etc. Yoon, S-D reported that the ascorbic acid inclusion improves the thermal properties of starch-PVA polymer films. And it is found that ascorbic acid is better than poly-ols in getting films with good thermal properties (Yoon 2014).

Chitosan

With numerous biological and therapeutic uses, chitosan is another resourceful natural biopolymer. Chitin and its deacetylated derivative chitosan are natural biopolymers composed of *N*-acetyl-D-glucosamine (acetylated unit) and β -(1-4)-linked D-glucosamine (deacetylated unit) connected in a random manner (Rinaudo 2006). Deacetylating of chitin by hydrolyzing the acetamide groups with concentrated NaOH or KOH at temperatures above 100 °C produces chitosan. This reaction occurs in a heterogeneous environment most of the time. It is a white powder that dissolves in 1% acetic acid and has a particle size of less than 120 mesh. Chitosan can link to negatively charged molecules because of the presence of positive amine groups. It does, however, have several disadvantages, such as low mechanical strength and a slow processing speed. In applications based on polymer materials, mechanical properties of polymers and polymer mixes are most important, but their thermal properties must also be considered. The temperature at which polymers degrade must be determined before they may be used in thermal applications. This natural biopolymer contains amine groups in its structure, has a positive charge, and can thus bond to negatively charged molecules (Fig. 4).

Chitosan is used in a variety of industries, including biotechnology, pharmaceuticals, environmental chemistry, and tissue engineering (Kumar et al. 2004). Unlike cellulose, chitosan is a simple polysaccharide with distinct characteristics that may be easily tweaked through functionalization techniques such as N-acylation, production of aldimines and ketimines, interaction with keto-acids, and N-carboxymethylation. Due to the ability of its amino groups to function as chelation sites, it can adsorb a range of toxic metals (Jha et al. 1988). For the majority of heavy metals, chitosan-based sorbents have demonstrated relatively strong sorption capacities and kinetics due to their high nitrogen content and porosity. Acid, vat, and

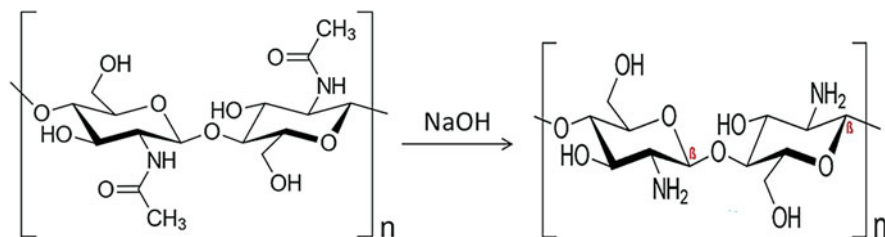


Fig. 4 Formation of chitosan from chitin

naphthol dyes have a strong affinity for chitosan (Shin et al. 2022). Another field in which chitosan can serve is in solid-state batteries in acid media due to its ionic conductivity (Ravi Kumar 2000), and it is also a suitable matrix for controlled medication release with minimal adverse effects. Chitosan hydrogels that are p^H -sensitive aid in medication delivery. Chitosan is used to make biodegradable wound dressing bandages because it inhibits microbial, fungal, and bacterial growth Chandy and Sharma (1990). Understanding their thermal stability and pyrolysis could help them comprehend and organize their industrial processes more effectively.

According to Emilia Szymańska and Winnicka (2015), chitosan is quite sensitive to environmental factors, so it is best to keep it in well-sealed containers at low temperatures (2–8 °C). It is critical to undertake stability tests during the preparation process of chitosan-based applications to determine the product's shelf-life. Chitosan (medium molecular weight), Aldrich, had a melting point of around 290 °C. It is determined by the degree of deacetylation as well as the molecular weight (10,000–1,000,000 g/mol). It is an amorphous material with a T_g value of 203 °C. Chitosan is extremely sensitive to a variety of degradation processes, including thermo-degradation. It can be biodegraded into harmless residues, with the amount of degradation being inversely proportional to the molecular weight of the substance. Understanding their thermal stability and pyrolysis could aid in better understanding and planning their industrial processes.

Chitosan is comparable in structure and properties to glycosaminoglycan (GAG), a natural polysaccharide that is a main constituent of the external matrix of cells. The degree of deacetylation is found to tune the properties like molecular weight, solubility, and viscosity. Chitosan deacetylation ranges from 50% to 100%, with the latter being entirely deacetylated chitin. Chitosan has a molar mass of 50–2000 kDa on an average. The structural analysis of chitosan produced from shrimp exoskeleton was carried out by E. Onsosen and O. Skaugrud (1990) using several instrumental techniques including FTIR, XRD, and SEM analysis, and the key features are listed below in Table 2 and Fig. 5.

Despite the fact that chitosan is a biocompatible polymer, it has a low heat resistance. Heat is frequently used in the creation of medication formulations for delivery of drugs. High temperature exposure can alter the structure of a polymer, influencing its solubility and viscosity. The temperature at which glass transition occurs for the chitosan moieties was observed to move to a larger value with an increase in its molecular mass, indicating that thermal degradation of the chitosan structure is a complicated process, which involving two or more decomposition steps. Based on these findings, it may be concluded that chitosan can be heated

Table 2 Structural analyses data of chitosan from FT-IR and XRD

FTIR data	Wavenumber (cm ⁻¹)	XRD data (2θ)
O-H, N-H stretching	3444.87	9.63
C-H stretching	2920.23	20.53
C-O stretching	1398.39	
N-H bending characteristic of chitosan polysaccharide	1635.64	

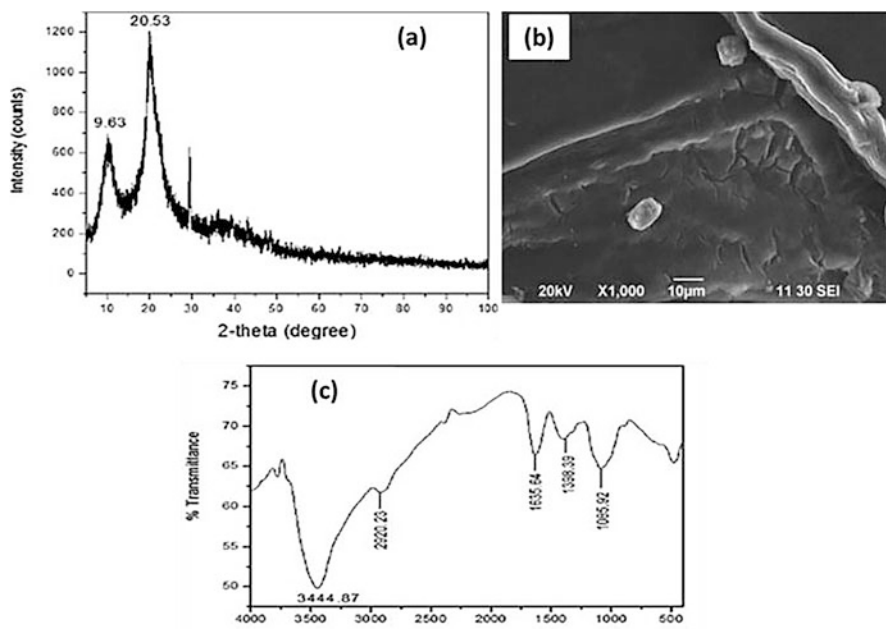


Fig. 5 Structural analyses of chitosan from XRD, SEM, and FT-IR. (Adapted from Dey et al. 2016)

below its glass transition temperature without losing its physicochemical features. The loss of water as a result of thermal treatment is thought to be the cause of lowering the T_g , making the material more temperature-dependent, and, as a result, reducing its storage steadiness. In chitosan-based systems, the inclusion of medication, plasticizer, or other additives tends to lower the polymer glass transition temperature. Tissue engineering (Croiser and Jerome 2013) is one of the most important usages for chitosan polymer blends. Novel chitosan/polyol-phosphate compositions have received numerous applications in the biomedical field due to the altered chitosan's ability to become heat sensitive in diluted acids and go through gelation at body temperature. These characteristics make it an appealing tool for a variety of uses, including local drug injecting systems and sophisticated tissue-engineering vehicles. Some of the chitosan thermal investigations have been described, indicating that there is still room for improvement in the thermal stability of the most cost-effective biopolymer.

Thermal Analysis of Chitosan: Effect of Various Factors

Thermogravimetric analysis and differential scanning calorimetric experiment results are summarized here. Thermogravimetric research offers accurate data on physicochemical parameters, solid process transformation, and solid participation in

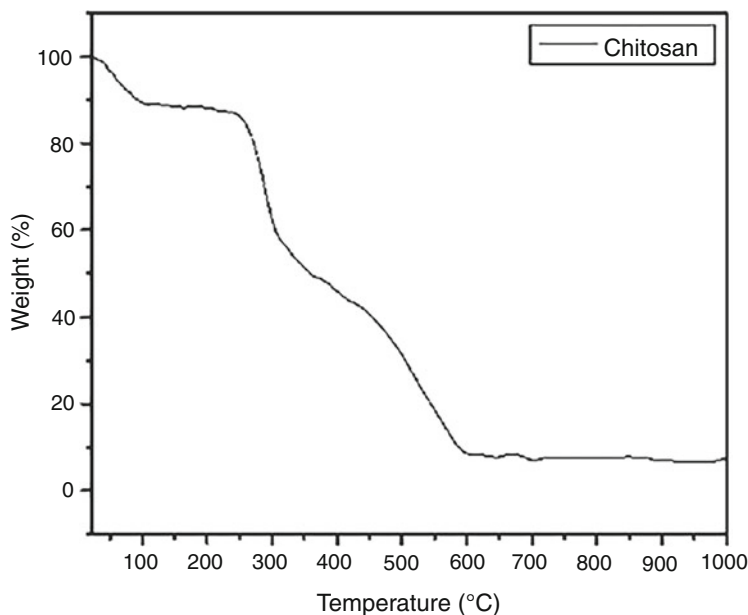


Fig. 6 TGA of chitosan. (Adapted from Dey et al. 2016)

isothermal and nonisothermal heating. According to studies by Nieto et al. (1991), there were two stages in the TG plot (Fig. 6).

The analysis shows a moisture loss in the temperature range of 22–100 °C, and vaporization and removal of volatile products occur at 248–600 °C. The polysaccharide glycosidic linkages in chitosan are randomly split during pyrolysis and converted into various fatty acids. According to DSC analysis, it is observed that an endothermic peak occurs at 77–80 °C which is caused by the loss of water bonded with hydrophilic groups. A second peak around 303–304 °C is an exothermic peak which occurs due to chitosan network degradation.

The nonisothermal ultrasound-assisted deacetylation process (NIUSAD) was used to produce chitosan samples with a variety of characteristics, such as an average degree of acetylation ranging from 7.5% to 25.0%, an average molecular weight measured from viscosity measurements that varied from $1.17 \times 10^5 \text{ g mol}^{-1}$ to $6.45 \times 10^5 \text{ g mol}^{-1}$ and crystallinity index average (19.1%– CI -52.7%). The findings showed that each of these variables has an impact on the thermal stability of NIUSAD chitosan, with the crystallinity index having the greatest influence on the temperature at which deterioration really starts.

Several investigations on chitosan and its modification have revealed that a number of parameters influence heat deterioration (Georgieva et al. 2012). One such factor is the rate at which the sample is heated. The effect of the rate of heating on thermal degradation was performed by Peng-Zhi Hong et al. They used thermogravimetric analysis to investigate the temperature-dependent breakdown of

chitosan at various rate of heating in nitrogen. The findings suggest that chitosan thermal breakdown in nitrogen is a one-step process. Temperatures of deterioration rise as rate of heating(B) increases. The temperature at the maximum rate of degradation, or the peak temperature on a differential thermogravimetry curve (T_p), is $1.291B + 355.2$ °C, and the temperature at which the maximum rate of degradation ends (T_f) is $1.505B + 369.7$ °C. B has little effect on the strongest thermal degradation reactions at T_p and T_f , which have average values of 50.17% and 72.16%, respectively. The peak height on a differential thermogravimetry curve (R_p), which represents the greatest thermal degradation reaction rate, rises with B. The frequency factor, A, and activation energy for the reaction, E, dramatically rise during the reaction's initial phase, then it remains largely constant before reaching a peak value during the final phase. The thermal degradation's rate constants alter with the level of decomposition and rise as the reaction temperature rises. The variation in the decomposition curve with heating rate is illustrated in Fig. 7.

The temperature of maximal reaction rate for the first stage of thermal degradation increased from 294 °C to 328 °C in another investigation by Douglas de Britto et al., when the heating rate was changed from 2.5 °C/min to 15 °C/min. It was also discovered that E_a (Activation energy) is independent of the heating method used,

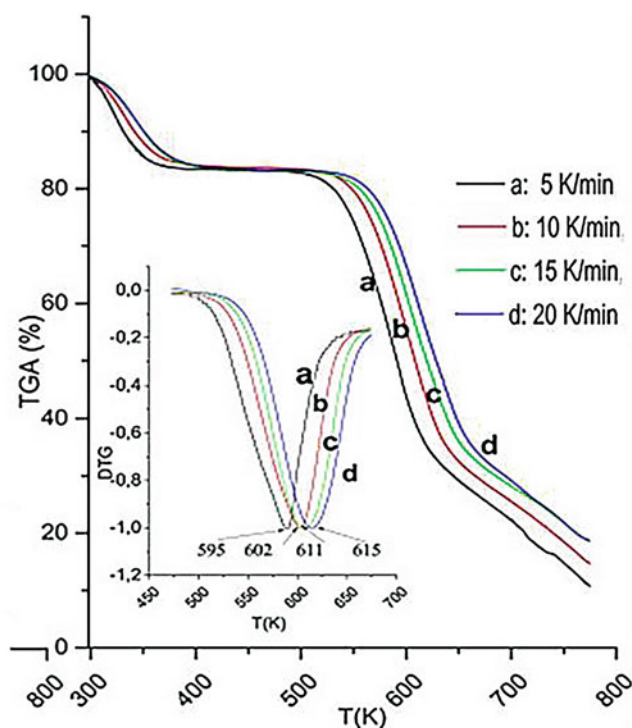


Fig. 7 TG curves of the thermal degradation of chitosan at heating rates (a) 5 K/min, (b) 10 K/min, (c) 15 K/min, and (d) 20 K/min. (Adapted from Moussout et al. 2016)

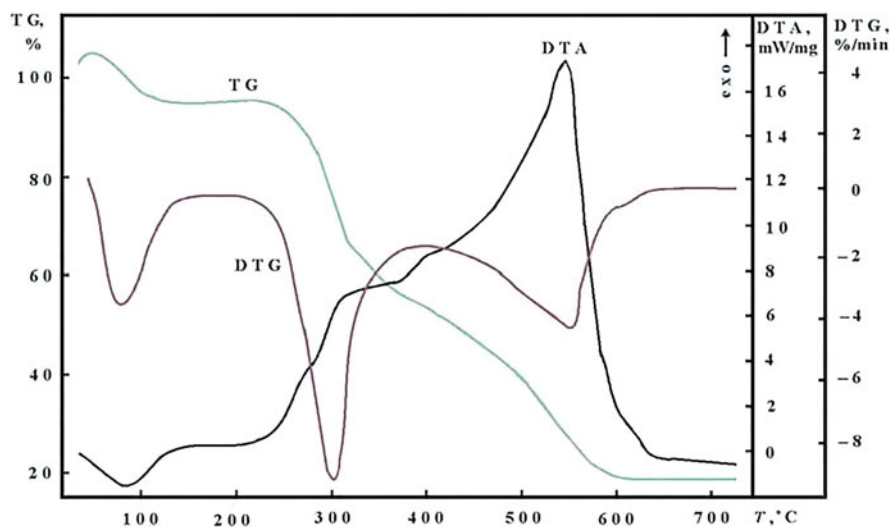


Fig. 8 TG, DTG, and DTA curves, obtained for thermal degradation of chitosan at heating rate of $15\text{ }^{\circ}\text{C min}^{-1}$. (Adapted from reference Georgieva et al. 2012)

whether it is isothermal or dynamic. However, it has been hypothesized that it may be dependent on the average degree of deacetylation and the polymer's physical state (powder or film). Depolymerization and deacetylation were found to have an effect on thermal degradation as observed by significant changes in the TG-curve at temperatures exceeding $380\text{ }^{\circ}\text{C}$. It could be due to a change in the material's structure as well as a change in the mechanism of its thermal degradation process. Two peaks were seen in the DTG and DTA curves, at 295 and $545\text{ }^{\circ}\text{C}$, respectively. Both of these steps are highly exothermic. The first stage, which ends at $400\text{ }^{\circ}\text{C}$, is associated with a weight loss of 43.5% , whereas the next stage is associated with a mass loss of 35% . The first stage, according to some writers Hong et al. (2007), is associated with chitosan deacetylation and depolymerization. The second step is due to a degradation of residual cross-links in polymer (Fig. 8).

An investigation on the effect of different acid solvents on thermal behavior of chitosan films was evaluated by Hugo Yves et al. (2019). The impact of different acids on the thermal characteristics of chitosan films was studied. Polymer solutions of $100\text{ }\mu\text{m}$ thick films were created in a variety of acids, such as hydrochloric, nitric, and organic acids like tartaric, acetic, citric, lactic, and maleic acids. Infrared spectroscopy, X-ray diffraction, and thermal analysis were used to examine the films. They were less crystalline and more clear than chitosan powder. The intra- and intermolecular interactions in chitosan films were altered by the acid's nature, as evidenced by the shift to higher frequencies of the bands at $2942\text{--}2784\text{ cm}^{-1}$ and the shift to lower frequencies of the bands at 1650 , 1590 , 1420 , and 1376 cm^{-1} in the infrared spectra. Films had lesser thermal stability than chitosan, and each acid was affecting the thermal stability differently (Fig. 9).

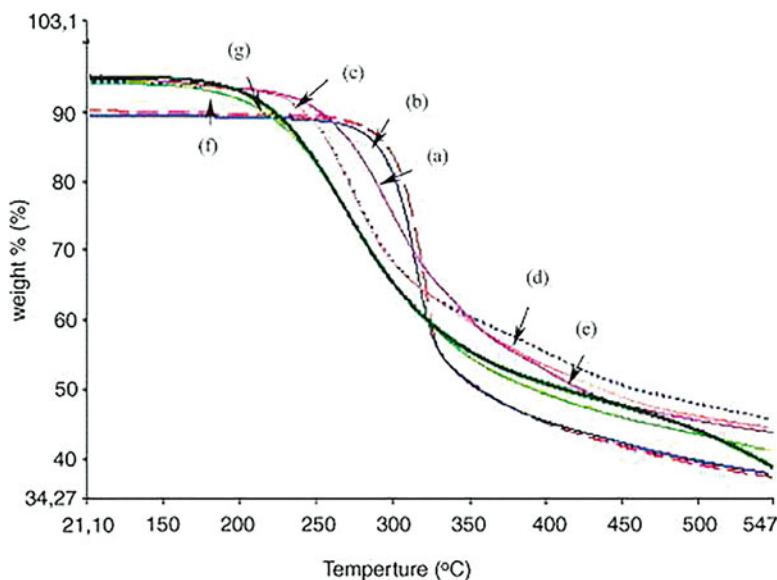


Fig. 9 Degradation behavior of different molecular mass of chitosans measured by thermo gravimetric analysis: (a) 400 kDa, (b) 250 kDa, (c) 100 kDa, (d) 50 kDa, (e) 25 kDa, (f) 5 kDa, and (g) 2.5 kDa. (Adapted from reference Mao et al. 2004)

Degradation temperature was dependent on molecular weight according to the observation made on chitosan of different molecular weights. According to S. Mao et al., molecular weight is a factor to consider while studying thermal behavior. Low molecular mass chitosan decomposes at a low temperature than large molecular weight chitosan. Chitosan of molecular weight of 250–500 kDa showed a highest degradation temperature of roughly 280 °C. 100–250 MW requires 220 °C and MW 25–100 and 2.5–5 kDa is 180 °C. Chitosan solubility increased as molecular weight decreased, and chitosan breakdown temperature was also affected by molecular weight.

Methods to Improve the Thermal Stability

Certain applications for which polymers have been used thermal stability are major criteria. One method to improve the stability is to blend it with polymer. The commonly used polymers are starch, poly(vinyl alcohol), poly(ethyleneoxide), and polyvinylpyrrolidone. A study carried out on using these polymers as blends showed that blending with PVA (Mao et al. 2004) increased the moisture stability and this can be due to higher structural packing attained due to cross-linking. According to the study conducted on PVA-chitosan blends (Cui et al. 2018) for drug delivery application, it was seen that two endothermic peaks were observed at 160 °C and 220 °C and shifted to higher temperatures. The shift to a higher temperature of the

first peak is the replacement of the amino and hydroxyl group of chitosan by crosslinking agents. This shows that PVA cross-linked with chitosan was a better option than the composite of PVA- chitosan for high temperature applications. In comparison to pure chitosan, PVA addition on chitosan/polyvinylpyrrolidone films was found to have a higher initial temperature of thermal decomposition.

When a natural polymer, such as carboxymethyl cellulose (Thomas et al. 2016), is blended with glycerol and formaldehyde, the thermal stability is improved. When comparing pure chitosan to chitosan cross-linked with formaldehyde, it is clear that the weight loss (percent) of chitosan cross-linked with formaldehyde was larger. The temperature at which pure chitosan degrades is 120 °C. Chitosan cross-linked with 0.2% formaldehyde had the best thermal stability up to 220 °C. When chitosan is combined with an excessive amount of crosslinking agent, a hydrogel is formed. When compared to pure CMC, CMC crosslinked with formaldehyde improves thermal stability. The temperature at which pure CMC degrades is 240 °C. At 260 °C, CMC crosslinked with 0.4% formaldehyde provided the best thermal stability.

In order to study the thermal properties of cross-linking, studies were conducted on chitosan, chitosan cross-linked with glutaraldehyde, and blended poly (ethyleneoxide) polymer. It was found the on cross-linking and blending the thermal stability was improved. It was found that there was more hydrophilicity for the cross linked chitosan and thermal stability decreased. According to Zhang et al. (2021), combining CS and polycaprolactone at the optimal ratio of 9:1 significantly improved the membranes' thermal stability, hydrophilicity, and dynamic mechanical viscoelasticity. By using dynamic mechanical thermal analysis, all of the samples in the experimental range showed strong elasticity at low temperatures and high viscosity at high temperatures (DMTA). The glass transition temperature of polycaprolactone increased from 64.8 to 76 and the initial degradation temperature increased up to 86 °C. The most significant variation was seen in crystallinity and porosity which increased up to 30% and 86%. The elongation on breakage was 200%. So these properties make this membrane blend suitable for tissue engineering. Further studies were performed by adding acrylic acid along with poly caprolactone to chitosan (PCL-g-AA). The compatibility of PCL-g-AA with chitosan and, subsequently, the characteristics of the blend were both significantly improved as a result of the production of ester and imide groups, which improved the dispersion and homogeneity of chitosan in the matrix. Furthermore, PCL-g-AA/chitosan was easier to process than PCL/chitosan because it had a lower melt temperature. The PCL-g-AA/chitosan combination had greater water resistance, which also affected how resistant it was to degrading in soil and in an enzymatic environment. However, weight loss of the blends after being buried in soil or exposed to an enzymatic environment suggests that both blends were biodegradable, particularly at high chitosan concentration levels. When polyamide was blended in, the thermal stability was also enhanced.

The cross linkage methodology applied can be chemical or physical factors. Usually chemical cross linking increases the stability due to covalent bonding and hydrophilic and hydrophobic interaction. The cross-linkers used are glutaraldehyde,

cinnamic acid, and glyoxal. Three component chitosan/sodium alginate/pentasodium tripolyphosphate (Ch/NaAlg/TPP) membranes, as well as one component chitosan (Ch), two component chitosan/sodium alginate (Ch/NaAlg), and chitosan/pentasodium tripolyphosphate (Ch/TPP) membranes, were examined. It was shown from the above study that the thermal stability increases in the following order: Ch/NaAlg < Ch/NaAlg/TPP < Ch/TPPNaAlgChTPP if the temperature at which thermal deterioration begins is used as a criterion for the thermal stability. A study on the chemical reaction between chitosan and cinnamic acid showed that the stability of the polymer was decreased (Dieb et.al. 2012). On reacting cinnamic acid with chitosan gave the corresponding N-cinnamoyl chitosan (NCC) polymer. NCC polymer was having less thermal stability when compared to chitosan polymer according to a thermogravimetric study. Using the Arrhenius relationship, the activation energies for heat degradation of chitosan and NCC polymers were calculated. Research on the heat degradation of NCC polymer revealed that the removal of low-molecular-weight radicals is the mechanism through which NCC polymer degrades and this might be the reason for low heat stability.

With advancements in nanochemistry, attempts have been made to improve chitosan's thermal and mechanical properties by incorporation of nanoparticles. The main advantage of chitosan is that it is a cost-effective matrix with a biocompatible character that may be used to create hybrid products. Silica is one of the cost effective material, which can be used in combination with chitosan to prepare biosensors and catalyst and also find application in analytical field. It can easily be prepared using sol-gel method. Narrower distribution of particles and reduced particle size and development of oxide of the metal in situ leads to improved strength and increased glass transition temperature. The hydroxyl and amino groups in chitosan helps in the formation of gel network. The chitosan dissolved in 2% HAG and hydrolysis of TEOS resulted in the formation of gel network. The % of silica was varied and flexible chitosan-silica hybrids were produced. Characteristic absorption in FTIR corresponding to N-H stretching (3249 cm^{-1}) showed a decrease in intensity and shift to lesser frequency and showed more interfacial interaction between chitosan matrix and inorganic phase. A new absorption band at 960 cm^{-1} related Si-OH due to H-bonding to amide and oxy group of chitosan. The particle size of silica was 2 to 7 nm. Thermogravimetric investigation revealed that the first stage weight loss between $100\text{ }^{\circ}\text{C}$ and $160\text{ }^{\circ}\text{C}$ appears to be caused by the loss of absorbed water on the surface of chitosan as well as a side product of the subsequent condensation of the Si-OH groups (Al-sageer and Muslim 2010). Because low molecular weight species decompose more slowly between $160\text{ }^{\circ}\text{C}$ and $270\text{ }^{\circ}\text{C}$, chitosan loses weight more slowly. The complex dehydration of the saccharide rings, depolymerization, and thermal degradation of the acetylated and deacetylated units of the polymer all occur in the range between $170\text{ }^{\circ}\text{C}$ and $450\text{ }^{\circ}\text{C}$. The silica network's incorporation and interaction with the polymer raise the hybrids' thermal resistance, which also raises the temperature at which they decompose thermally. The mass of residue retained at $750\text{ }^{\circ}\text{C}$ indicates the amount of silica content incorporated in the hybrids. The displacement in the glass transition temperature

(T_g) of the composites from the maxima of the transition curves has been measured using dynamic mechanical thermal analysis (DMTA). As the silica content rises, so do the storage modulus and glass transition temperature. With 30 weight percent silica, the T_g value rises to its highest point, which is 159.37 °C.

A number of properties of chitosan have been modified by addition of nano-fillers of metal oxide. Metal oxides such ZnO, Fe₃O₄, and TiO₂ were found to increase the magnetic properties and tensile properties. The studies using MgO (De Silva et al. 2017), which is prepared easily from economical raw materials like brine, magnesium salts, etc., are limited. In a study a new magnesium carbonate/poly (methylmethacrylate) (PMMA) composite precursor was successfully heat-treated to produce spherical MgO nanoparticles for the first time. Chitosan-MgO composites were synthesized by solution casting technique. TG analysis showed that there were three mass loss stages. First was around 100 °C due to absorbed moisture, which was followed by the second mass loss at 200–300 °C which could be due to the thermal decomposition of amine and -CH₂OH groups of chitosan, and the final step at 525–650 °C could be due to the degradation of the glucosamine ring. The addition of MgO to chitosan films increased their thermal stability substantially. For example, the degradation temperature of pure chitosan film is 325 °C at 50% mass loss, but it has shifted to 450 °C and 529 °C with the addition of 5 and 10 (w/w percent) MgO, which is a 27% and 59% increase, respectively. This increase has been attributed to the high dispersion of MgO which hinders the diffusion and the great thermal stability of MgO above 250 °C as well as the homogeneous dispersion of MgO could explain these improvements.

The effect of nanoalumina (Aziz et al. 2011) on the glass transition was studied on films of chitosan and its complexes prepared using solution-casting technique. Varying amounts of Al₂O₃ (3, 4.5, 6, 7.5, 9, 12, and 15 wt%) were added to the highest room temperature conducting sample in the chitosan–salt system, that is, sample containing 60 wt% chitosan–40 wt% NH₄SCN. The glass transition temperature was studied using differential scanning calorimetry and T_g for the highest conducting composite is 190 °C. The increase in T_g with increase in more than 6 wt % filler content is attributed to the increase in degree of crystallinity. Thus, research is still going on to improve the thermal stability of chitosan, the most versatile natural polymer.

Polylactic Acid (PLA)

Polylactic acid (PLA), also known as the first generation biobased biodegradable polyester, is the most widely used biopolymer and is originated from renewable materials like sugar and corn starch. In the food packaging sector, tissue scaffolding, and biomedical devices, the aliphatic polyester polylactic acid (PLA) (Scheme 1), made from lactic acid (2-hydroxypropionic acid), finds widespread use. The PLA monomer lactic acid comes in two optical isomeric structures: Levorotary or L isomer and Dextrorotary or D isomer, which results in the polymers PLLA and

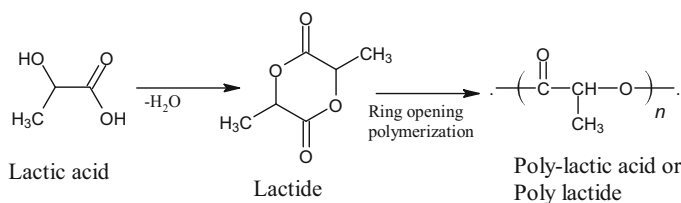


Fig. 10 Production of PLA

PDLA (Wuisman and Smit 2006). PLA is a particularly desirable material to employ in substitution of petroleum-based polymers, because of its strong mechanical qualities and good processability. However, PLA is hydrophobic in nature with low thermal stability, sluggish to degrade, poor toughness, and less reactive side chain groups (Barkhad et al. 2020). PLA has a wide variety of applications which includes automotive parts, mechanical parts, electrical and electronic devices, and materials for 3D printing, etc. (Fig. 10).

PLA can be produced by sensibly controlling the polymerization of its D- and L-isomers to obtain highly crystalline and amorphous polymers with high molecular weight distribution which is generally recognized as safe for food packaging applications. As this polymer readily undergoes hydrolysis, no major processing techniques can be used to produce them in large scales and for that, high molecular weight polymers with good thermal stability are required.

Thermal Properties of PLA

The thermal degradation temperature of PLA starts around 200 °C according to the thermo gravimetric (TGA) analysis and is caused by a variety of factors, including reformation of the lactide structure, hydrolysis, oxidative scission of the polymer chain, etc. However, there have been reports of two degradation phases for PLA, at 300 °C and 340 °C, as a result of the stereoisomeric structure (D- and L-forms) of the polymer, which can affect the polymer's thermal characteristics (Aoyagi et al. 2002). According to Varol's TGA analysis, the degradation temperatures of PLLA and PDLA pellets were found to be 296 °C and 321 °C respectively (Fig. 11). The average molecular weight of the two PLAs, 53 and 56 kDa for PLLA and PDLA respectively, explains this difference (Varol 2019) (Fig. 11).

The melting temperature (T_m) and glass transition temperature (T_g) of PLA significantly affect the physical-chemical stability of foods in food packaging applications. The possibility of heat-treated packaging of PLA is very less as the T_m of PLA is low. Homopolymers of PLA showed a glass transition temperature of around 60 °C, the specific heat capacity C_p is 0.51 J/(g °C), and the melt temperature is around 175 °C.

Poly(lactid acid) is a slower-crystallizing material; however, pure PLA crystallizes at the highest rates, producing crystalline spherulitic morphology in the

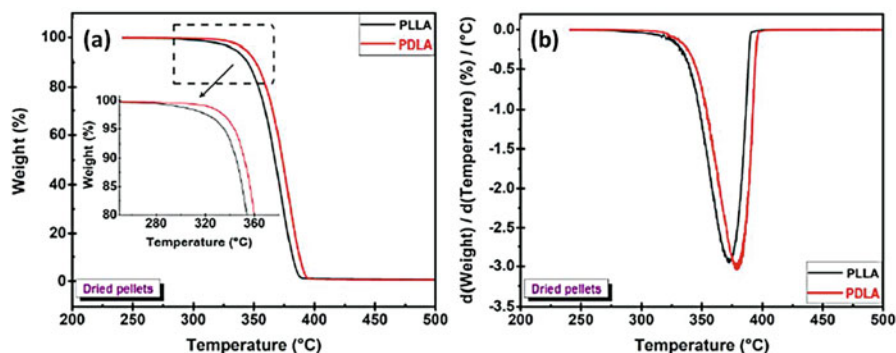


Fig. 11 TGA curves of PLLA and PDLA (a) thermograms and (b) derivative curves as a function of temperature. (Adapted from reference Varol 2019)

temperature range of 110–130 °C. The impact of the co-monomer length, its content, and architectural characteristics on the PLA crystallization should also be taken into account for the copolymers based on PLA. In general, the crystallization process is related to the nuclei formation and consequent crystal growth. For commercial applications, a specific amount of crystallinity is necessary; however, it is difficult to produce high crystallinity. A variety of formulations or modifications are essential to fasten the PLA's crystallization. Some of them are addition of nucleating agents for inducing crystallization at higher temperatures, use of plasticizers to increase the segmental mobility by reducing the chain folding energy, and also the production of the stereocomplex crystallites of the enantiomeric forms of PLA. In recent years, the last formulation, that is, the making of stereocomplex crystallites, has got wide attention since they have superior properties compared to the D- and L- forms of PLA. PLA exists in four diverse crystalline forms α , β , γ , and δ based on the different crystallization conditions and the PLA composition. Recently, stereocomplex crystallites received much attention and the melting temperature of the same is about 50 °C higher than that of PLA homochiral crystallites. Peaks for X-ray diffraction can be seen in the most typical form that crystallizes from melt or solution at 14.8°, 16.9°, 19.1°, and 22.5°. The disordered α form crystal or γ form crystal is generated below 120 °C and has comparable diffraction patterns to the α form crystals.

The controls of processing parameters on the thermal insulation behavior of melt-processed PLA revealed a considerable rise in the degree of crystallization with an increase in annealing time, which had a direct impact on the glass transition temperature, density, and thermal conductivity. Typically, PLA's thermal properties were established by the Carslaw and Jaeger mathematical model, and this model was for a real heat source with definite power and negligible losses. The thermal conductance (λ) is expressed using the equation, $\lambda = \frac{P}{\Delta T} \frac{h}{S}$, where P is the power of the electric heat source, S is its surface area, h is the distance from the heat source to the temperature sensor, and ΔT is the temperature difference between them.

Thermal Behavior of Blends of PLA with Other Polymers

Numerous studies on PLA blends have been conducted in an effort to enhance some PLA characteristics, such as crystallinity index, biodegradability, or mechanical properties for packaging use. The Gordon–Taylor equation, $Tg^b = \frac{W_1 Tg_1 + k W_2 Tg_2}{W_1 + k W_2}$, where Tg^b , Tg_1 , and Tg_2 are the glass transition temperatures of the blend, and the neat components, W is the weight fraction, and k is a parameter that can be adjusted and is a measure of miscibility which can be correlated to the strength of interaction between the individual blend components.

The thermal behavior of PLA/starch blends that have been annealed at various temperatures was compared by Shanshan and coworkers (Shanshan et al. 2015). It was discovered that annealing helped to reduce or perhaps completely eradicate the enthalpy relaxation at Tg . DSC was used to determine the degree of crystallinity (X_c), using the equation, $X_c = \frac{1}{W_f} \left[\frac{\Delta H_m - \Delta H_c}{\Delta H_{m0}} \right]$, where ΔH_m is the enthalpy for melting, ΔH_c is the enthalpy for cold crystallization, ΔH_{m0} is the melting enthalpy of the 100% crystalline PLA, and W_f is the weight fraction of PLA in the composites. The findings show that with increase in the annealing temperatures, the samples' crystallinity gets elevated. It was established that during the annealing process, the disordered crystal had a tendency to transform into the ordered crystal. In addition to the behavior showed by pure PLA, all of the PLA/starch blends also demonstrated a double melting behavior. The lower melting temperature ($Tm1$) increased as the annealing temperature was elevated, while the $Tm2$ did not significantly alter. The XRD patterns additionally demonstrated that annealing helped the materials to increase crystallinity. The thermal stability of the annealed samples was much less than that of the virgin samples which was supported by the activation energy values obtained from the Flynn–Wall–Ozawa method.

Similar to PLA, the biopolyester polyhydroxybutyrate (PHB) is also produced through bacterial fermentation from renewable resources like corn or sugar. Even though PLA and PHB's low thermal stability restricts their commercial applications, introducing PHB to PLA has been proven to increase PLA's mechanical strength and crystallinity (Armentano et al. 2015). In their review, Kervran and coworkers reported that in contrast to PHB, which has stronger thermal stability in PLA/PHB blend, PLA showed a poor thermal stability in the PLA/PHB blend than the pure PLA (Kervran et al. 2022). Dynamic study of the PLA/PHB blends TGA curves showed that the blends degraded in two stages. PHB degradation was thought to be responsible for the first stage of mass loss, and PLA was thought to be responsible for the second (Aydemir and Gardner 2020).

Several studies investigated the PLA systems, by adding additives such as cellulose-based reinforcements, plasticizers, crosslinking agents, etc., to increase the mechanical characteristics, flexibility, and physicochemical properties of the PLA/PHB blends for improved performance in superior applications (Trusca and Panaitescu 2019). Different plasticizers, comprising acetyl tributyl citrate (ATBC), oligomeric lactic acid, vegetable oils, and polyethylene glycol, are employed to plasticize PLA/PHB blends. However, the dynamic analyses revealed that the

addition of ATBC had no appreciable impact on the T_{\max} values of PLA and PHB. In comparison to lower molecular weight polyethylene glycol (PEG) (Mw 4000), the higher molecular weight PEG (Mw 6000) showed better thermal stability in PHB/PLA blends. So the thermal stability of PHB/PLA can be varied by the addition of polyethylene glycol with different molecular weights (Panaitescu et al. 2017). A similar improvement in PHB's thermal stability was seen when epoxidized soybean oil (ESBO) or epoxidized linseed oil were added to vegetable oils used for plasticizing PLA/PHB blends (Garcia et al. 2016; Lopera-Valle et al. 2019). This may be accounted for by the fact that vegetable oils may enhance the interaction between their molecular fragments and the polymeric backbones, resulting in the development of a thin physical barrier on the surface of the material and a subsequent reduction in the permeability of volatile substances. Additionally, the capacity of the epoxidized vegetable oils to scavenge acid groups during the breakdown improves the thermal stability. In the same way, in order to establish bonds between the chains of PHB and PLA polymeric chains, many works were reported showing the use of many crosslinking agents, such as maleic anhydride (MA), dicumyl peroxide (DCP). Based on different interpretations, inserting MA in the PLA/PHB blends can serve as a bridge by forming covalent connections by dipole-dipole interaction, hydrogen bonding or transesterification reaction, which leads to the enhanced interactions of PLA and PHB chains. While just a modest improvement in thermal characteristics was seen with no appreciable change in T_{\max} , this could improve the mechanical as well as morphological aspects. Frone and coworkers noticed that adding 1 wt.% of DCP to a 75/25 PLA/PHB blend did not significantly alter the T_{\max} values, but that the T_{onset} and $T_{10\%}$ values decreased for the blends with and without DCP by 5 °C and 17 °C, respectively. When MA and DCP were evaluated on PLA/PHB blends, the first one improved the blend's thermal stability, especially the thermal stability of the PHB in the blends, while the second one decreased the $T_{10\%}$ and T_{\max} values (Frone et al. 2019). Graphene oxide (GO)-based crosslinker was prepared with PLA in a very recent research by Sujana and coworkers, and a modest improvement in thermal stability was noted, which may be related to the interfacial contacts between the GO-based crosslinker and the polymer chains. The TGA, DTG, and DSC analyses all showed that the inclusion of GO-LA crosslinker increased the thermal stability of GO cross linked PLA (Sujana et al. 2021).

Extruded films are seen as alternatives to conventional synthetic-polymer films because of their exceptional mechanical and thermal properties and immediate prospective applications in extrusion-method large-scale manufacture of biodegradable polymers. Castillo et al. produced films made of polypropylene, poly(lactic acid), and chitosan using a solvent-free extrusion technique, using glycerol and polypropylene-graft maleic anhydride as plasticizer and compatibilizer, respectively. The thermal and mechanical qualities of extruded films containing up to 50% by weight of biopolymers are acceptable, and the addition of a compatibilizer enhances the processing features (Castillo et al. 2020).

In addition to that a lot of researchers reported the incorporation of agricultural fibers into PLA which could enhance the flame retardancy and thermal stability of the fiber reinforced PLA and which varies according to the kind and pretreatment

technique of the agricultural fibers used in creating fiber-reinforced PLA. (Yiga et al. 2021). Tej Singh et al. explored the consequences of *Dalbergia sissoo* wood waste on the glass transition and cold crystallization temperatures of the PLA-based composites, whereas with the increase of wood waste content, the crystallinity was found to be increasing after a first reduction in the value (Singh et al. 2021). A similar work recently reported to evaluate the feasibility of wood leachate (WL) constituents especially phenol and lignin as an additive in PLA-based biocomposites, and they reported that the thermal stability of PLA and its crystallization behavior were found to be improved with the addition of WL as a filler (Shahavi et al. 2022).

The fire resistance and UV stability properties of PLA and its composites are becoming significant because of their relevance in commercial applications. The mechanical and fire resistance of the PLA matrix was significantly enhanced by the addition of nano-ZnO and cellulose nanocrystals (CNC) to the polymer matrix. The studies confirmed that ZnO nanoparticles can display fire-retardant qualities, and the 1.5% CNC and 1.5% ZnO nanocomposites outperformed all others in terms of having the least amount of mass loss. Due to substantial char generation, oxidative combustion did not exhibit a clearly defined single decomposition, indicating two distinct decomposition processes. The shielding effect from ZnO acts as an insulator and causes noncontinuous burning, which boosts the fire retardancy of nanocomposites in addition to the use of CNCs (Bajwa et al. 2021).

Due to their low weight and simplicity of production, thermally conductive polymer composites are frequently employed as heat dissipation materials in LEDs and electronic packaging. Typically, high thermal conductivity fillers like metal, ceramic, and carbon are incorporated into a polymer matrix to create thermally conductive nanocomposites.

Conclusion

In many industries, biodegradable polymers have emerged as superior substitutes for traditional polymers. It is ascribed to their simplicity in production, low cost, and ease in degradation. The majority of biopolymers used in implants has electrical components and/or possess temperature-dependent properties that are essential for interacting with biological tissues. Being directly tied to morphology and having a direct impact on a material's therapeutic performance, a material's thermal behavior plays a significant role in characterizing its properties.

Based on quantitative and qualitative thermal assessments, this chapter covers some of the thermal characteristics of four pertinent biopolymers that were most extensively explored in the literature. Relying on apparent heat capacity, quantitative thermal analyses of semicrystalline and amorphous biopolymers were carried out and the findings were interpreted in terms of the molecular mobility in the microscopic level throughout the whole temperature range, including several transition zones. The facts presented in this chapter were carried out using the conventional DSC, conventional TMDSC, adiabatic calorimetry, quasi-isothermal TMDSC, etc. The discrete phases and phase transitions, such as the melt and cold crystallization,

the enthalpy of relaxation, crystal rearrangement, melting, glass transition, etc., could be characterized by these thermal analyses. Quantitative measurements gave an idea about the amount of crystalline, amorphous, and semicrystalline fractions. With the inclusion of nanocellulose, the thermal properties of nanocomposites have typically improved. Since nanocellulose degrades at a temperature of about 200–300 °C, processing temperatures should be kept below that range. The two primary issues that still exist with cellulose are its ability to break down into nanoscale particles and its compatibility with other polymeric materials. Similar to cellulose, derivatization or the creation of composites can be used to alter the thermal characteristics of starch. Esterification of hydroxyl groups of starch will help in improving the thermal stability. Blended composites of starch with various plasticizers and other additives exhibit variable thermal properties. Chitosan and PLA also have restricted applications due to its poor thermal stability. To enhance the thermal characteristics, the utilization of cross-linking agents, nanofillers, blending with polymers, and several other methods were applied and some are illustrated in this chapter.

In a nutshell, it is possible to improve the thermal conductivity of bio-based polymers by incorporating various structural parameters. In particular, structural modification through noncovalent functionalization offers an effective method for creating nanocomposites with high thermal conductivity, which makes them highly desirable in a variety of heat management applications, including lighting systems, the automotive industry, and next-generation microelectronics.

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Gas Barrier Properties of Biopolymers

12

Neena George and B. Venugopal

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Abstract

Growing environmental consciousness has made the development of biobased, biodegradable, high-performance packaging materials increasingly popular. Biobased packaging materials, as opposed to those made from fossil carbon, constitute a solution that can significantly improve the environment because of their sustainability, biodegradability, biocompatibility, availability, and lack of toxicity. Since the passage of oxygen or moisture can cause the contents of packaging to degrade, gas barrier qualities are essential. Various criteria used to evaluate the barrier properties, evaluation methods, and methods utilized to enhance gas barrier properties of packaging films are covered in this chapter. A few significant biopolymers' oxygen and water vapor barrier characteristics are thoroughly explored. Recent studies on the use of cellulose, starch, and pectin, as

N. George (✉) · B. Venugopal

Post Graduate and Research Department of Chemistry, Maharaja's College, Ernakulam, Kerala, India

e-mail: neenageorge@maharajas.ac.in

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natural biopolymers in barrier films, have been discussed. A focus has been placed on the barrier qualities of synthetic biopolymers such polyhydroxybutyrate (PHB), poly (3-hydroxybutyrate-co-3-hydroxyvalerate), polyvinyl alcohol (PVA), and polylactic acid (PLA). This chapter also provides a broad overview of how these biopolymers work with other substances to produce balanced qualities suitable for barrier packaging.

Keywords

Gas barrier · Biopolymer · Packaging · Permeability

Introduction

The primary goal of a package especially food package is to safeguard the goods from the environment while also fulfilling this objective over the long haul. Food taste, quality, shelf life, and marketability can all be significantly influenced by food packaging. The usage of high-quality packaging, which limits transmission of oxygen and moisture between objects and the environment, is thought to have a key role in preventing food spoiling. A significant factor in food deterioration is the loss or gain of oxygen and water. Making airtight seals and using superior barrier materials are two methods for enhancing packaging in order to extend the shelf life of items. To assist increasing the food's ability to be stored, especially if it is likely to go rancid, some packages contain desiccants, oxygen absorbers, or scavengers. New developments in this field include the use of gas barrier films to control gas permeation.

Gas barrier films for the packaging of food, drugs, and electronics utilize petroleum-derived synthetic polymers. Since almost everything one buys, comes in a package, globalization has caused packaging to have an impact on every part of the industry. The majority of plastic packing films are used once, then burned or dumped in landfills. Due to the fact that these synthetic polymers are neither carbon neutral nor biodegradable, this results in environmental issues. Alternatives made of biopolymers to petroleum-based materials have the potential to cut carbon dioxide emissions by 30–70%, hence lowering the global carbon footprint (Greene 2014).

Biodegradable barrier materials should be used more heavily in packaging materials, which currently make up 60% of plastic items, to reduce the environmental risks. But still a number of issues are being faced by biobased barriers, such as its relatively increased cost, low resistance to heat, water, gases, and mechanical strain, which severely restrict the use of conventional biodegradable polymers for food packaging. This chapter discusses the gas barrier properties of biopolymers under two classes. Starch-, cellulose-, and pectin-based barrier films have been discussed under natural biopolymers. Polyhydroxybutyrates (PHB), Polylactic acid (PLA), and Polyvinyl alcohol (PVA) based barriers are discussed under synthetic biopolymers.

Gas Barrier Property in Polymers

Gas barriers are devices that can impede or stop the flow of gases and vapors. What is kept behind the barrier is usually protected by restricting gas passage. The gas barrier qualities are crucial in polymers used for food packaging because even extremely small amounts of oxygen can cause most products to degrade. Gas barriers are also used in the packaging of commodities like pharmaceutical, electrical, and medical products. No substance is capable of creating an endless barrier to gases.

In polymers, gas permeation is determined by the concentration gradient of the diffusing gas. Gas molecules have a natural tendency to flow across the barrier depending on the concentration gradient. The mechanism is determined by the barrier material and gas' intrinsic physical qualities, as well as the temperature circumstances. For a barrier film, gas permeation includes three steps: (i) gas dissolution into the film, (ii) diffusion through the film, and (iii) desorption from the film. Among this diffusion through the film is the rate-determining step.

Table 1 categorizes the most popular polymers used in packaging applications as barriers based on oxygen and water vapor permeability. From the table, it is clear that oxygen barrier property is opposite to the moisture barrier behavior. This is because good oxygen barriers are always associated with polar bonds or hydrogen bonds within their molecular structure which results in high hydrophilicity (Lagaron et al. 2004). Table 1 gives only rough values since the barrier value of the material will vary depending on the nature, source, grade, and processing methods employed.

Table 1 Classification of polymer films on the basis of oxygen and water vapor permeability^a (Wang et al. 2018)

Grade	Oxygen permeability (cm ³ · μm. m ⁻² day ⁻¹ atm ⁻¹)	Example	WVP (g. μm. m ⁻² day ⁻¹ kPa ⁻¹	Example
Poor	>40,000	HDPE, PP,PS, PHB,LDPE	>3000	Silicone Eastomer
Low	4000–40,000	PVC, BOPP, PHA, PLA	1000–3000	PA 6, PLA
Medium	400–4000	EVOH wet, OPET, PA 6 wet, PET	400–1000	PS, PHA, PLA
High	40–400	PVdC, PA6 dry	40–400	PAN, PVC, PET, LDPE
Very high	<40	EVOH dry	<40	HDPE, PP, PVdC, OPET, BOPP

^aOP values at 23–25 °C without specified RH% and WVP values at 37.8 °C and 90% relative humidity (RH). *BOPP* biaxially oriented polypropylene, *EVOH* ethylene vinyl alcohol, *HDPE* high-density polyethylene, *LDPE* low-density polyethylene, *OPET* oriented polyethylene terephthalate, *PA 6* polyamide 6, *PET* polyethylene terephthalate, *PHA* polyhydroxyalkanoates, *PHB* polyhydroxybutyrate, *PLA* polylactic acid, *PP* polypropylene, *PVC* polyvinyl chloride, *PVdC* polyvinylidene chloride, *PS* polystyrene, and *PAN* polyacrylonitrile

Measuring Barrier Properties

There are various terms to express a material's barrier properties, including transmission rate, permeability, and permeance. The transmission rate is defined as the amount a permeant permeates a film per unit surface area per unit time. Permeance is the transmission rate multiplied by the difference in partial pressure of the permeant. The permeability is equal to the permeance times the thickness. Temperature, pressure, and relative humidity are additional factors that affect a material's barrier capabilities in addition to the characteristics of the material itself. In a controlled setting, equilibrium moisture conditions are typically used to test barrier qualities. A hygroscopic material typically needs one or two days to reach equilibrium. Precision in the controlling test conditions and constancy in sample preparation determine accuracy and reproducibility. The relative humidity across the test film, the partial pressure of the permeant, and the thickness of the film all affect the transmission rate and permeance. Even if it is standardized to thickness, a material's permeability may differ if measured at two distinct thicknesses. At a particular temperature and relative humidity, the property is measured at 1 atm in most cases. Even if a publication only lists one of the parameters, these can be transformed into each other using test conditions and known film thickness.

Barrier performance of a packaging material can be expressed in terms of *Permeability*. SI unit of permeability is $\text{mol m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$, or $\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$. Barrer is a non-SI unit of gas permeability (Eq. 1).

$$1 \text{ barrer} = 10^{-10} \frac{\text{cm}_{\text{STP}}^3 \cdot \text{cm}}{\text{cm}^2 \cdot \text{s} \cdot \text{cm Hg}} \quad (1)$$

where ' cm_{STP}^3 ' represents the amount of gas (moles or molecules) that occupies one cc at STP, "cm" represents the thickness of the test material, $\text{cm}_{\text{STP}}^3 \text{cm}^{-2} \text{s}^{-1}$ represents the flux of gas that flows through the material, and "cmHg" stands for the pressure drop across the material.

Gas transmission, such as oxygen, liquid and vapor transmission, and oil transmission are the most typical barrier measurements. The characteristics of the penetrating molecule, those of the film material, and the environment all affect the barrier attributes. Feeding the gas into one side of the sample cell and using a sweep gas to convey the penetrated gas to the detector is one method of measuring gas permeation. The parameters of the measurement significantly affect the result. It is necessary to measure, monitor, and record the difference in temperature and humidity across the sample. Comparison of two results would be useless if the measurement conditions were unknown.

Some of the common types of barrier parameters are given below.

Water vapor permeability can be expressed either in terms of **water vapor transmission rate (WVTR)** or **water vapor permeability (WVP)**. WVTR is defined as the mass of water vapor or moisture that permeates per surface area of the barrier film for a definite time period, temperature, and pressure gradient (Rastogi and Samyn 2015).

The standard ASTM E96M-16 is typically used to measure WVTR and WVP (Abdullah et al. 2019). The procedure makes use of a borosilicate glass bottle with a mouth of 3 cm dia, filled with distilled water, and sealed with the sample. To prevent any contact, keep at least a 20 mm space between the water and the samples. Keep the temperature and relative humidity (RH) steady. For instance, an RH gradient of 100/50% can be achieved by utilizing purified water within the container (i.e., 100% RH) and saturated solution of $\text{Mg}(\text{NO}_3)_2 \cdot 6 \text{H}_2\text{O}$ outside it (i.e., 50% RH). To keep the conditions almost identical, the cups are kept in an air oven. Loss in weight of the cups should be noted every day for 2 weeks. WVTR is calculated using the following equation (Eq. 2)

$$\text{WVTR} = \frac{\text{Weight passed through}}{\text{Area} \times \text{Time}} = \frac{\frac{W}{t}}{A} \quad (2)$$

where W, t, W/t, and A stand for weight loss (g), time (h), the slope of linear regression (g/h), and the film area (i.e., cup mouth area) (m^2), respectively. SI unit of WVTR is $\text{g m}^{-2} \text{day}^{-1}$.

WVP of samples can be calculated according to the following equation (Eq. 3):

$$\text{WVP} = \frac{\text{WVTR} \times \text{thickness}}{\text{Saturated pressure} \times \Delta\% \text{RH}} = \frac{\text{WVTR} \times l}{S \times \Delta \text{RH}} \quad (3)$$

where l is the film thickness in “m,” S is saturated vapor pressure of water in “kPa,” and ΔRH is relative humidity gradient (for example, 100/50% in this case). SI unit of WVP is $\text{g } \mu\text{m m}^{-2} \text{day}^{-1} \text{ kPa}^{-1}$.

Since oxygen is the most reactive gas in the atmosphere, it is crucial to shield food material from it in order to ensure a long shelf life. Oxygen transmission can be measured in a number of methods. As per ASTM D3985-17 (2017) standard, the most conventional oxygen transfer rate (OTR) measurement uses the MOCON instrument. OTR is the amount of oxygen that travels through a barrier sample in a specific amount of time under steady-state conditions (Eq. 4).

$$\text{OTR} = \frac{\text{Volume passed through}}{\text{Area} \times \text{time}} = \frac{\frac{V}{t}}{A} \quad (4)$$

SI unit of OTR is $\text{cm}^3 \text{m}^{-2} \text{day}^{-1}$.

The barrier material is positioned between two chambers in this technique under dry conditions (RH 1%) and at standard atmospheric pressure. Both oxygen and nitrogen gas are stored in separate chambers. A coulometric detector detects the penetrated oxygen (Siracusa et al. 2008).

In a conventional gas permeability tester, the preconditioned sample film was placed between two teflon rings in a permeation chamber (temperature and relative humidity should be kept constant and monitored). N_2 gas was used as a carrier gas in one chamber of the permeation cell while O_2 /air was circulated at a controlled flow rate to maintain the pressure gradient of equal to 80 kPa in the other chamber.

The gas mixture was directly injected into a gas chromatograph that was connected to a thermal conductivity detector for examination after a delay of around 1 h. The column temperature was kept at 50 °C, and the used helium flow rate was set at 25 ml/min. O₂/air and N₂ peaks were used to calculate the final test gas content.

Oxygen permeability (OP) is calculated as per the equation below (Eq. 5).

$$OP = \frac{OTR \times \text{Film thickness}}{\text{Oxygen partial pressure difference}} \quad (5)$$

SI unit of OP is cm³ μm m⁻² day⁻¹ atm⁻¹.

Transmission rate of other gases can be calculated once that of oxygen is done using their relationship. Nitrogen has a transmission rate one-third that of oxygen, whereas carbon dioxide has a transmission rate six times that of oxygen. The *carbon dioxide transmission rate* (CO₂TR) is the measure of the amount of CO₂ gas that flows across a substance in a specific amount of time, and it is measured in cc m⁻² s⁻¹. CO₂TR, like WVTR and OTR, plays an important role in food packaging (Siracusa et al. 2008).

Natural Biopolymers

In-depth research is being done on the use of polysaccharides as food-packaging films, including starches, cellulose, chitosan, and pectin. Unfortunately, the poor mechanical properties, high WVP, and moisture sensitivity of polysaccharide derivatives prevent them from being used in industrial applications (Zhang et al. 2014). Some of the recent studies which make use of polysaccharides such as starch, cellulose, and pectin in film applications are discussed below.

Starch

One of the natural resources that is easily obtainable from a variety of renewable sources and may thus be generated in big amounts is starch. At the end of its life cycle, it is fully biodegradable and also quite affordable. It is composed of the same 1, 4-α-D-glucopyranosyl units that make up amylose and amylopectin. Amylose is linear with repeating units connected through α (1–4) connections, whereas amylopectin has a backbone that is α (1–4) linked and contains around 5% α (1–6) linked branches. The relative proportions of these two vary depending on the plant origin and determine the functional properties of starch. Starches with a high amylose percentage can create films that are fracture-resistant (Mali et al. 2004). Aside from carbohydrates, starch granules also include trace amounts of lipids, proteins, and minerals (Rindlav-Westling et al. 1998).

The starch granules are insoluble in cold water in their natural form, hence the majority of uses need for a heating process with an abundance of water. Granules of starch enlarge significantly after absorbing water, below a certain temperature, or the

so-called gelatinization temperature, of roughly 60 °C. The diffusion of amylose outside the granule is responsible for this process. If the temperature goes up to this limit, the inflated starch granules may break apart into smaller aggregates or particles, which will produce gelatinized starch. Thermoplastic starch, also known as plasticized starch, is made when native starch is fragmented and plasticized using water and another plasticizer such as glycerol (Fringant et al. 1996; Majdzadeh-Ardakani et al. 2010). But in native form, its application is constrained by its hydrophilic nature, and poor mechanical and barrier performance.

Chemically Modified Starch Films

A typical method for enhancing the properties of starch films is chemically altering the starch to add new functional moieties that can reduce solubility and WVP. This can also result in improved mechanical properties. By adding varied amounts of glycerol, Cyras et al. (2006) studied the characteristics of gelatinized potato starch and discovered that the glycerol content of the films influences the material's behavior at room temperature.

His research showed that the strong hydrogen bonding between chains of starch and glycerol causes some loss in the crystallinity of the films on glycerol addition. Additionally, the researchers included several chemical alterations in their investigation, including acetylation (using acetic acid and anhydride) and maleation (using maleic anhydride). Due to the decreased contact between the starch chains with reduced -OH groups following alteration, these modifications significantly decreased crystallinity. Esterification of starch reduces water sorption in contrast to that of neat starch because acetylated and maleated starch has a less hydrophilic character than native starch.

According to Zamudio-Flores et al. (2006), oxidized banana starch can be utilized to produce films with better barrier properties. In their study, glycerol and oxidized banana starch were used to create the films. Oxidized banana starch was made at three different oxidation levels (active chlorine concentrations of 0.5, 1.0, and 1.5%). The use of thermal gelatinization and casting was done to prepare the film. Tensile properties, XRD pattern, and water vapor transmission rates of these materials were examined. The films' tensile strength increased together with the degree of oxidation of the banana starch. The peak crystallinity of the films grew together with the starch's level of oxidation. The mechanical strength of the films was improved by the stronger bonds between polymer chains and between polymer and glycerol molecules. The films maintained for 30–60 days did not exhibit any statistically significant variations in their water vapor permeability (WVPc). The highest WVP were found in the films made from oxidized banana starch, and these values improved as the starch's level of oxidation increased.

According to López et al. (2008), acetylated maize starch had the best properties for creating films, was more transparent, and was easier to deal with. Four treatments of modified corn starch viz. acetylated, interwoven/acetylated, interwoven/hydroxypropylated, and acid hydrolysis were used in this study. WVP was decreased in all of the corn starch films in addition of functional groups.

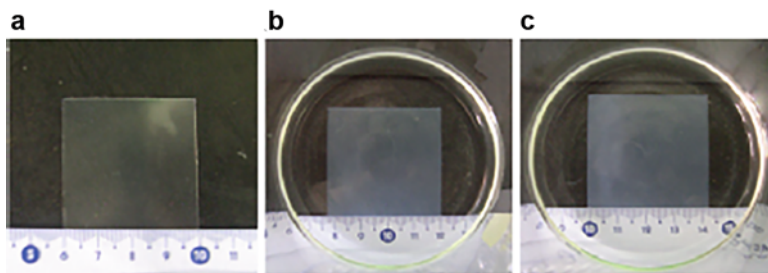


Fig. 1 Photographs of oxidized potato starch films in acidic condition: (a) before test; (b) 1 day; and (c) 60 days

Using oxidized potato starch (OPS) and glycerol as a plasticizer at various concentrations, Hu et al. (2009) made starch films. The OPS films were pliable and transparent. The film with 19.4% glycerol appeared to have desirable mechanical qualities, and the crystallinity of OPS films decreased as glycerol content increased. The film's resistance to crosslinking, resistance to leaking, and stability in acidic or basic conditions were also studied. The results revealed that the OPS films exhibited excellent antileakage properties for vegetable oil, good anti-crosslinking properties in saturated formaldehyde vapor, and high stability in acidic aqueous medium, but low stability in alkali aqueous medium (Fig. 1).

Starch-Based Moisture-Proof Films

When using starch films to wrap meals with high moisture content or those are stored under highly humid conditions, the ability of the film to withstand moisture is a very important film quality. To effectively transmit moisture from wrapped foods to the film matrix, stretchy films must strongly adhere to the food surface. Therefore, the real use of stretchable starch films in the food business depends greatly on their moisture-proofing ability. Starch film's effective moisture-proofing ability preserved the intended texture of the food product by preventing water interaction with the environment, when it was used to wrap dry meals (Yang and Paulson 2000). The moisture-proof property of the film is crucial for preserving the anticipated film performance when it comes to wrapping fresh goods with high moisture content (Petersen et al. 1999).

Using equally mixed octenylsuccinated/native starches (OSS/NS), Li et al. (2015) were successful in creating stretchable sweet potato starch films with better moisture-proof properties. The OSS/NS blend films greatly outperformed the control film with respect to moisture resistance, as evidenced by decreased water solubility and WVP and increased contact angle and oil permeability. The water solubility and WVP of OSS/NS mix films decreased by 23.7% and 20.8%, respectively, at a degree of substitution (DS) of 0.0425. Elongation at break improved by 84.5%, and contact angle and oil permeability improved by 181.1% and 43.6%, respectively. The amplitudes of these gains changed as OSS substitution levels (DS) increased. The OSS/NS blend films' stretchability, elongation at break, and transparency were all

high and enhanced as the DS of OSS increased. OSS/NS mix films displayed less crystallinity than the control film, as evident from XRD measurements. The hydrophobicity of OSS and the plasticization of octenyl groups during film production were essentially responsible for these discrepancies.

Starch Blends in Barrier Applications

Other methods include adding nanoparticles, blending starch with other macromolecules or polymers (such as pectin, cellulose fibers, or proteins), or both. Nanoclays were found to improve the mechanical properties and reduce WVP of cassava starch films as reported by Souza et al. (2012). Using polylactic-acid, Sanyang et al. (2016) attempted to enhance the functional qualities of sugar palm starch (SPS)-based films (PLA). The inclusion of PLA boosts tensile strength and tensile modulus while reducing water uptake. Overall, adding a PLA layer to SPS films makes them stronger mechanically and more water-resistant, which increases their suitability for food packaging.

Ryberg et al. (2011) created renewable barrier films from a wood hydrolysate. By including a co-component, such as CMC or chitosan, the interactions among the constituent molecules are tailored to achieve the right balance. The barriers, which were coated on a PET substrate, displayed extremely low OP at relative humidity levels of 50 and 80% and showed worse results than coatings manufactured from O-acetyl galactoglucomannan, a highly purified form of spruce hemicellulose (AcGGM). They claim that a wood hydrolysate/CMC coating is the optimal composition since it lowers the OP of PET from $14.6 \text{ cm}^3 \mu\text{m}^{-2} \text{ day}^{-1} \text{ kPa}^{-1}$ to as low as $1.6 \text{ cm}^3 \mu\text{m}^{-2} \text{ day}^{-1} \text{ kPa}^{-1}$.

Cellulose

Anselme Payen, a French scientist, discovered cellulose in 1838, and Hermann Staudinger described its polymer structure in 1920. As a polysaccharide, cellulose has the formula $(\text{C}_6\text{H}_{10}\text{O}_5)_n$. The hierarchical structure of cellulose is given in Fig. 1. Cellulose is made up of glucose monomer units. It is a linear chain made up of $\beta(1-4)$ glycosidic connections connecting D-anhydroglucopyranose units (Markovic and Visakh 2017). The noncellulosic materials usually found along with cellulose include lignin, pectin, hemicelluloses, proteins, fats, etc. Cellulose comes in four different crystalline forms, including cellulose I, II, III, and IV. Typically, cellulose I and cellulose II are found in plants (Habibi et al. 2010) in which thermodynamically most stable structure is cellulose II. A noncrystalline form of cellulose also exists, which is known as nematic ordered cellulose.

The polymer chains in cellulose are linear which gives them rigidity and rod-like nature. Intra and intermolecular hydrogen bonding in these chains gives them highly ordered crystalline nature. Due to high crystallinity, these fibers have good strength and are insoluble in solvents. Cellulose swells by absorbing water, but it does not dissolve in water.

Diffusing molecules typically have a hard time penetrating the crystalline portions of cellulose fibrils. Due to its relatively high crystallinity and the ability of the nanofibers to organize into a dense network that is held together by strong interfibrillar connections, it has been suggested that cellulose may serve as a barrier material. Since different cellulose and hemicellulose films offer strong resistance to oxygen absorption, they have been investigated as biobased oxygen barrier materials. For instance, Syverud and Stenius (2009) reported an oxygen transmission value for 21 μm -thick microfibrillated cellulose (MFC) films measured at 23 °C and 0% RH of $17.75 \pm 0.75 \text{ ml m}^{-2} \text{ day}^{-1}$.

When an MFC layer was applied to the PLA surface, observed that the oxygen permeability of polylactide (PLA) film decreased by a factor of more than 700. It is assumed that MFC has a tendency to absorb a substantial amount of moisture since it is very hydrophilic. The mesostructure of the films and the cellulose molecule structure are thought to regulate the intricate phenomena of MFC swelling and water absorption (Aulin et al. 2009).

Transparent, flexible regenerated cellulose films using aqueous alkali (NaOH or LiOH)/urea (AU) solutions were made by Yang et al. (2011). These films had higher oxygen barrier qualities than traditional cellophane, poly (vinylidene chloride), and poly (vinyl alcohol). From various cellulose sources, a series of AU cellulose films were created for various dissolution and regeneration environments. Depending on the conditions under which the films were made, the OP values of these films varied from 0.003 to 0.03 $\text{mL } \mu\text{m m}^{-2} \text{ day}^{-1} \text{ kPa}^{-1}$ at 0% RH. The AU film made from a 6 wt% cellulose solution and regenerated with acetone at 0 °C had the lowest oxygen permeability. It was discovered that the AU films produced by regeneration in a solvent at low temperatures from solutions with high cellulose content typically exhibited low oxygen permeabilities.

Table 2 details the characteristics and oxygen permeabilities (at 0% R.H.) of the cellulose films that were regenerated from aqueous AU solutions. The cellulose samples included filter paper pulp (FP), Whatman CF11 microcrystalline cellulose powder, another cotton linters pulp (CP), and softwood bleached kraft pulp (SBKP).

From Table 2, it is clear that OP value of the AU cellulose film is dependent on the nature of solvent used and regeneration procedure. The lowest value of oxygen permeability reported in this study was $0.003 \text{ mL } \mu\text{m m}^{-2} \text{ day}^{-1} \text{ kPa}^{-1}$ for the film from a 6 wt % cellulose solution in LiOH/urea/H₂O by regeneration with acetone at 0 °C. According to the study, no plastic film has ever been known to have an oxygen permeability as high as this AU cellulose film. For instance, the oxygen permeabilities of nylon-6, PVC, HDPE, PET, and PVA are, respectively, 6, 0.4, 500, 8, and $0.04 \text{ mL } \mu\text{mm}^{-2} \text{ day}^{-1} \text{ kPa}^{-1}$ at 0% R.H. The AU cellulose films also offer good bendability, strong mechanical strengths, and great optical transparency.

Figure 2 shows how OP values and densities of cellulose films made from the solutions by regeneration with 5 wt% H₂SO₄/5 wt % Na₂SO₄ are affected by the concentration of FP cellulose in LiOH/urea/H₂O solutions. The regeneration temperature in this instance was set at 20 °C. The OP of the films decreased from 0.03 to $0.012 \text{ mL } \mu\text{m m}^{-2} \text{ day}^{-1} \text{ kPa}^{-1}$ when the cellulose content was raised from 3 to 6 wt %. In consequence, the AU cellulose sheets' density rose from 1.39 to 1.45 g cm^{-3} .

Table 2 Preparation conditions of AU cellulose solutions and characteristics of regenerated AU cellulose films prepared from them. (Adapted with permission from reference (Yang et al. 2011))

Solvent	Cellulose source	C (wt %)	Regeneration bath	T (°C)	Density (gcm ⁻³)	Oxygen permeability (mL μm m ⁻² day ⁻¹ kPa ⁻¹)
NaOH/urea	FP	5	5% H ₂ SO ₄	0	1.54	0.0058
NaOH/urea	FP	5	5% H ₂ SO ₄	20	1.40	0.0185
NaOH/urea	FP	5	5% H ₂ SO ₄	60	1.35	0.0304
NaOH/urea	FP	5	5% H ₂ SO ₄ and 5% Na ₂ SO ₄	20	1.45	0.0140
LiOH/urea	FP	3	5% H ₂ SO ₄ and 5% Na ₂ SO ₄	20	1.39	0.0296
LiOH/urea	FP	4	5% H ₂ SO ₄ and 5% Na ₂ SO ₄	20	1.43	0.0153
LiOH/urea	FP	5	5% H ₂ SO ₄ and 5% Na ₂ SO ₄	20	1.44	0.0137
LiOH/urea	FP	6	5% H ₂ SO ₄ and 5% Na ₂ SO ₄	20	1.45	0.0124
LiOH/urea	FP	6	EtOH	20	1.49	0.0109
LiOH/urea	FP	6	t-BuOH	20	1.51	0.0084
LiOH/urea	FP	6	Acetone	20	1.55	0.0045
LiOH/urea	FP	6	Acetone	0	1.55	0.0033
LiOH/urea	CF11	6	5% H ₂ SO ₄ and 5% Na ₂ SO ₄	20	1.38	0.0196
LiOH/urea	CP	4	5% H ₂ SO ₄ and 5% Na ₂ SO ₄	20	1.44	0.0149
LiOH/urea	SBKP	4	5% H ₂ SO ₄ and 5% Na ₂ SO ₄	20	1.45	0.0145
Cellophane					1.35	0.0481

FP filter paper pulp, CF11 cellulose powder, CP cotton linters pulp, SBKP softwood bleached kraft pulp. C is cellulose concentration in the AU solution. T is the temperature of the regeneration bath. Oxygen permeabilities of the films were measured at 0% R.H.

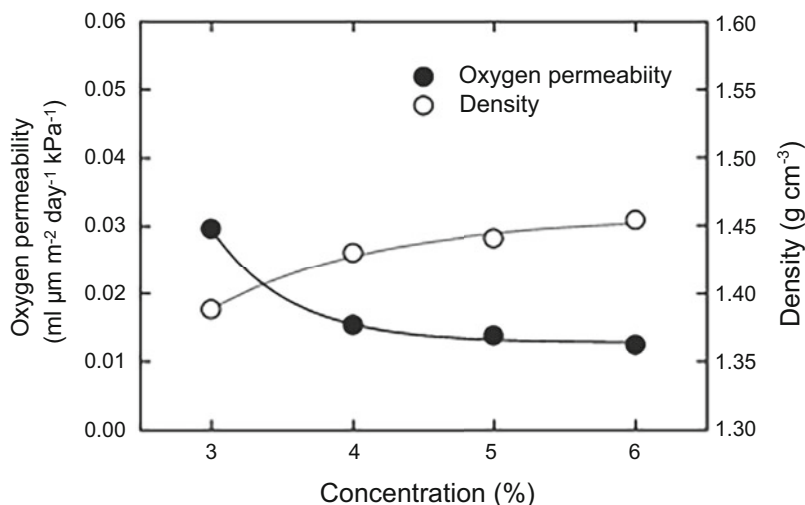


Fig. 2 Effects of FP cellulose concentration of LiOH/urea/H₂O solutions on oxygen permeabilities and densities of cellulose films prepared from solutions by regeneration with 5% H₂SO₄/ 5% Na₂SO₄ at 20 °C. (Adapted with permission from reference (Yang et al. 2011))

Because they are biodegradable and environmentally friendly, these transparent, flexible, high oxygen barrier cellulose materials show promise as high-performance packaging materials.

Hartman et al. (2006) examined the film-forming capacity of hemicellulose *O*-acetyl-galactoglucomannan (AcGGM) and assessed the material's potential as a barrier. From industrial process water obtained from mechanically pulping wood, the AcGGM was separated and films were made by solution casting. The effects of plasticizers such glycerol, sorbitol, and xylitol were compared in order to prevent brittleness. These additions did, however, increase susceptibility to moisture, which might not be as advantageous for other applications. Interesting mechanical strength and oxygen barrier qualities were attained in a film made from a physical mixture of carboxymethylcellulose or alginate, both of which have a significantly larger molecular weight than AcGGM. Due to the abundance of hydroxyl functionalities in the materials used, no phase separation was noticed. AcGGM-based film with optimum properties was made on the addition of a plasticizer with lower permeability for sorbitol compared to other plasticizers like glycerol and xylitol.

A wood hydrolysate formed from the wastewater following the hydrothermal treatment of spruce wood was successfully used to prepare thin films and coatings, in another study (Edlund et al. 2010). This was improved using ultrafiltration to produce a noncellulosic fraction that was rich in oligo- and polysaccharides and contained 10% lignin relative to dry mass. Wood hydrolysate and CMC or chitosan mixes were used to make coatings for PET film and free-standing films by solution casting. Both kinds of combinations provide clear, glossy films and coatings with

good mechanical qualities and extremely low oxygen permeabilities which make them promising for packing.

Pectin

Pectin is the methylated ester of polygalacturonic acid with different degrees of methylation. It is a branched chain hetero-polysaccharide constituent of the plant cell walls in plants. Other polysaccharides have a known molecular weight, while pectin does not. With celluloses and hemicelluloses, it contributes to the construction of the cell. Commercially available pectin films have degrees of esterification between 15% and 25%. The degree of esterification affects the mechanical properties of pectin films. Samples with a higher esterification degree are more fragile (Penhasi and Median 2014).

Pectin is widely used in food and beverage industry as an additive (Willats et al. 2006). It is a food ingredient that is used in many different products as a thickening or colloidal stabilizer (Wang et al. 2021a). Pectin and its derived compounds are used as a conventional ingredient in functional foods. The degrees of methyl esterification of polysaccharide chain in pectin determine its gelling and film-forming properties. Thus the extent of methyl esterification is the deciding factor about its industrial applicability (Šešlija et al. 2018). Its peculiarity is its cytocompatibility, wound dressing, tissue engineering (Pereira et al. 2018), drug delivery (Almeida et al. 2017) etc., which are the different areas where pectin is exploited in the biomedical field (Kocaaga et al. 2019). Pectin is special because of its biocompatibility, edibility, biodegradability, ability to gel, and ability to form films, among other qualities. As a result, it is an excellent choice for creating food-packaging films (Vanitha and Khan 2019).

Different biodegradable packaging materials use pectin and its derivatives to act as a barrier for the packaged goods. Flexibility is the suitability of this material for considering it as a biodegradable packaging material. Pectin can be treated as the omnivorous diet and can be metabolized. This edible coating simultaneously preserves the nutritional value of packaged food products and shields them from enzymatic deterioration, flavor loss, lipid migration, and pathogenic attack control. Pectin film can be prepared very easily by spray coating, solvent casting, and knife coating. Still, pectin films have two disadvantages, poor thermophysical properties and inadequate mechanical performances, which restrict its applications in food-packaging industries (Espitia et al. 2014).

Low methoxyl (LM) pectin can create hard gels by cross-linking with calcium ions when the pH is low. LM pectin films are employed as edible coatings because these gels are exceptionally stable and capable of acting as water barriers. The capacity of edible films to incorporate a range of substances, including antioxidants, flavors, antibrowning agents, and antimicrobials, is one of its key features (Falguera et al. 2011). Mendes et al. investigated the barrier qualities of the composite made by integrating cocoa butter (CB) in a pectin matrix (PEC) (Mendes et al. 2020). According to a modified version of ASTM E96–16, these composite films' water

vapor barriers were examined. The water vapor transmission rate (WVTR) values were calculated. The force that propels water vapor through the film is the difference in relative humidity (RH). The fact that water vapor migrates through the hydrophilic portion of a film may have led to the prediction that adding hydrophobic substances (like CB) would diminish WVP, and the experimental results are consistent with the prediction. The WVPTR readings dropped from 2.0 to 0.7 g mm kPa⁻¹ h⁻¹ m⁻² as CB concentration rose. The kind, structure, and quantity of the added lipids will determine how successfully the water barrier qualities are improved (Helland et al. 2002). WVPTR may be decreased by the hydrophobic to hydrophilic fraction ratio in the composite film. Due to the hydrophobic nature of cocoa butter and uniform dispersion throughout the pectin matrix, water diffusion rates were decreased. In light of good WVPTR values, the PEC/CB films are suitable for use as moisture-resistant films in food packaging (Otoni et al. 2016).

It was discovered that avocados kept at 10 °C with edible pectin coatings on them had a lower oxygen transmission rate, which delayed fruit decay (Maftoonazad and Ramaswamy 2008). Moalemiyan et al. successfully prepared a coating film using pectin, sorbitol, and bee wax to preserve chopped mangoes in their natural state for more than 15 days (Moalemiyan et al. 2012).

Synthetic Biopolymers

The synthesis of high molecular weight of biopolymers is a tedious process. There can be further challenges. Because of the specific spatial arrangement of the natural biopolymers, synthesis of the copy of a natural polymer is not at all easy. But it is desirable to surpass these issues to overcome the low abundance of the natural biopolymers.

Poly Hydroxybutyrate (PHB)

One widely used biopolymer packaging material in industry is PLA. Among polyhydroxy alkanates (PHA), polyhydroxybutyrates (PHB) are considered a good candidate especially when incorporated with nanofillers (Keskin et al. 2017). PHB has mesophoric regions which is semicrystalline in nature. These regions are somewhat permeable to oxygen and water vapor and may result in deterioration of packaged food items. Thus, an ideal packaging material should be impervious to the atmosphere (Manikandan et al. 2020). Manikandan et al. studied the barrier properties of PHB-based graphene nanocomposite films and evaluated the role of graphene in preventing the transportation of oxygen and water vapor. In their research, adding hard graphene sheets (Gr-NP) to the polymer matrix improved the PHB's barrier qualities (PHB). The Gr-NP in the PHB matrix made the composite film impervious by converting the gas passage track in the composite more and more tortuous. This phenomenon is persisted only up to certain limit, higher concentration of Gr-NP resulted in agglomeration, and the tortuosity in the gas

transmission pathways may be lost. Oxygen (OP) and water vapor permeability (WVP) values of the neat PHB are $1.53 \text{ cm}^3 \text{ mm m}^{-2} \text{ d}^{-2} \text{ atm}^{-1}$ and $9.26 \text{ g m}^{-2} \text{ d}^{-2} \text{ atm}^{-1}$, respectively. On addition of 0.7 wt% of Gr-NPs, OP and WVP values of the PHB/Gr-NP composites were reduced to $0.4 \text{ cm}^3 \text{ mm m}^{-2} \text{ d}^{-2} \text{ atm}^{-1}$ and $4 \text{ g mm m}^{-2} \text{ d}^{-2} \text{ atm}^{-1}$, respectively and they found that it was the optimum filler loading. Further increase in Gr-NP resulted in filler agglomeration.

The OP and WVP values achieved at an ideal Gr-NP concentration in the composite were comparable to and, in some cases, better than those from related research that are referenced in the literature. The OP values of the composite films made of graphene oxide and the polymers such as amylopectin, polythene terephthalate, amylose, and PLA were 1.4, 1.3, 0.7 and $0.6 \text{ cm}^3 \text{ mm m}^{-2} \text{ d}^{-2} \text{ atm}^{-1}$, respectively (Goh et al. 2016). All these results are higher than the OP value ($0.4 \text{ cm}^3 \text{ mm m}^{-2} \text{ d}^{-2} \text{ atm}^{-1}$) obtained by Manikandan and coworkers in their study. The desired limit of OP value is $1.0 \text{ cm}^3 \text{ mm m}^{-2} \text{ d}^{-2} \text{ atm}^{-1}$ (Xu et al. 2020). WVP values showed similar trend in barrier properties compared to OP values.

Poly (3-Hydroxybutyrate-Co-3-Hydroxyvalerate) PHBV

The oxygen barrier characteristics of the PHBV/cellulose nanocrystal (CNC) composite were found to be improved by Malmir et al. Cellulose nanocrystals reduce the oxygen permeability of PHBV to a great extent. It happens as a result of CNC's favorable impact on nanoparticles' crystalline nature and the development of a more tortuous path for gas molecules in their morphology. They examined the nucleating effect of CNC on the crystallization of the PHBV matrix and demonstrated that good dispersion could be obtained up to 4 wt. % of CNC (Malmir et al. 2017).

Keratin/PHBV composites' barrier characteristics were researched by Pardo-Ibáñez et al. Melt blending was used to create composites with various keratin contents. The PHBV composite with the lowest oxygen permeability value contains 1% keratin. In comparison to pristine PHBV film, it dropped from 3×10^{-18} to $1 \times 10^{-18} \text{ m}^3 \text{ m m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$ for 1% keratin loading. Higher loadings have caused adverse effect on permeability. Homogeneity of the filler dispersion and hence the tortuosity is the reason for improved barrier property of the composite film. Depending on the size and shape of filler particles, the ideal content can fluctuate. Reduced crystallinity and agglomeration were the results of keratin loadings of more than 3% (Pardo-Ibáñez et al. 2014).

The impact of lignin in PHBV on the barrier qualities of carbon dioxide and oxygen was investigated by Kovalcik et al. The gas permeability was considerably decreased by incorporating 1 wt.% lignin with PHBV such as oxygen and carbon dioxide permeability has got reduced by 77% and by 91%, respectively, in comparison with the neat PHBV. The permeability coefficient for carbon dioxide is higher than that of oxygen in the case of PHBV composite, which is in accordance with gas permeability behavior of many polymers (Huglin and Zakaria 1983). The improved performance in the barrier property of the composite film is due to homogeneous dispersion of lignin in the matrix (Kovalcik et al. 2015).

Martinez-Sanz et al. examined and described nanocomposites constructed of PHBV and bacterial cellulose nanowhiskers (BCNW) with various BNCW concentrations (Martinez-Sanz et al. 2014). Barrier properties were improved with the incorporation of 1 wt. % BCNW. A very small quantity of the nanofillers, 1 wt. % BCNW, produce water permeability drop of 28%. They examined the oxygen and water vapor permeability behavior of PHBV samples which contained low [7.3 mol%, (PHBV7)] and high [40 mol %, (PHBV40)] valerate and discovered that the PHBV films' oxygen and water vapor barrier qualities drastically declined as valerate content rose. The oxygen permeability decreased from 8.16×10^{-19} to $5.24 \times 10^{-19} \text{ m}^3 \text{ m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$ for PHBV7 with 1% BCNW and from 10.76×10^{-19} to $9.45 \times 10^{-19} \text{ m}^3 \text{ m}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$ for PHBV40 and 1% BCNW composites.

Jost and Langowski looked into how different plasticizers affected the mechanical and barrier qualities of PHBV blends. Plasticizers made PHBV more crystalline and had a negative impact on mechanical qualities, although they improved barrier properties. Plasticizers containing ether and ketone groups found suitable for the preparation of PHBV nanocomposite films. Glycerol was shown to be the best plasticizer for the synthetic biopolymer PHBV in terms of oxygen and water vapor permeability (at 5 wt. %) (Jost and Langowski 2015).

The mechanical characteristics of PLA/PHBV blend films and their gas barrier features are being studied by Boufarguine et al. Compared to PLA, PHBV is superior in gas barrier properties. The composite is produced by multilayer coextrusion technique. When they evaluated the blends' helium permeability, they discovered that the presence of PHBV layers reduced it relative to neat PLA (Boufarguine et al. 2013). There are many benefits to helium permeability measurement. Since helium molecules are smaller than those of oxygen, conducting gas permeability studies now takes only less than an hour. As a neutral gas, helium has no interaction with the matrix of polymers. Zembouai et al. concluded from their research on the PLA/PHBV blend that the main cause of the enhancement of barrier characteristics is the crystalline nature of PHBV. As the PHBV ratio in the prepared mixtures increased, the barrier characteristics of the blends improved. This showed that PHBV is a good candidate to improve the barrier performance of PLA even at minimal proportions (Zembouai et al. 2013). Ambrosio-Martin et al. studied the mass transport behavior of PHBV-functionalized graphene sheets (FGS) composites produced by compression molding in a hot press. The oxygen permeability was analyzed. With FGS added, a decrease in oxygen permeability was observed. The PHBV/FGS composite with 3 wt. % FGS exhibited a decrease in permeability of 41.2% (Ambrosio-Martin et al. 2015).

Arturo Rodriguez et al. studied the oxygen permeability on biobased poly (butylene succinate)-PHBV/talc-starch composites (Rodriguez-Urbe et al. 2021). Because of the excellent barrier capabilities of PHBV and the hard, impermeable nature of talc, the barrier properties were significantly improved when these materials were used together with BioPBS. Due to better interfacial contact, the addition of a coupling agent (maleic anhydride grafted BioPBS/PHBV) substantially strengthened the oxygen barrier property. The hydrophilicity of the maleic anhydride groups in the compatibilizer is the main reason for the reduced water vapor barrier

property. The oxygen and water vapor penetration rate of the BioPBS in its neat form was significant, but it was reduced by around 68% in both cases, when 20 wt. % talc was added (oxygen and water permeability). This is due to the presence of stacked layers (platelets) of hydrated silicates ($\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$) inside talc which are impermeable to oxygen and water (Buzarovska et al. 2016). The lowest oxygen permeability and water vapor permeation rate were attained with 24% PHBV, 20% talc, and 1% MA-g-BioPBS/PHBV (80/20) in BioPBS, respectively. Because of its limited dispersion and hydrophilic nature, starch has a little impact on the oxygen and water vapor barrier characteristics.

Pal et al. evaluated the oxygen permeability and water vapor permeability of the compression-molded and cast-extruded PHBV/PBAT films according to the ASTM F3985 and ASTM F1249, respectively (Pal et al. 2020). The effect of nanoclay on the transfer rate of oxygen permeability of PHBV-/PBAT-based nanocomposite films were analyzed. For compression-molded film, oxygen permeability of PHBV/PBAT blend was $546.99 \text{ cm}^3 \text{ m}^{-2} \text{ day}^{-1} \text{ atm}^{-1}$, and reduction in permeability was improved by 54% after the addition of 1.2 wt. % nanoclay. The interface of PHBV and PBAT and the well-dispersed nanoclay helped the composite in reducing its oxygen permeability. The PHBV/PBAT/nanoclay nanocomposite films, prepared by film extrusion technique, showed 78.9% improvement in oxygen barrier property after the addition of 1.2 wt% nanoclay, which indicates that film extrusion technique is superior to compression molding for the preparation of gas barrier films. The reason for the composite film's improved oxygen barrier qualities may be due to the nanoclay's appropriate size, shape, and uniform dispersion in the matrix. Higher loading of nanoclay in the matrix resulted in agglomeration of the filler and hence the oxygen barrier properties get reduced.

The forced parallel orientation of films caused by the machine-directed arrangement of the polymer chains enhances the dispersion of nanoclay and, as a result, increases the crystallinity of the polymer composites. Compared to the compression-molding technique, the barrier qualities are improved with cast film extrusion. A similar pattern was seen in water vapor permeability also. Due to filler agglomeration, the addition of 1.8 wt% nanoclay to the PHBV/PBAT blend occasionally produced results that were lower and occasionally only slightly higher than the matrix. Mondal et al., on the other hand, improved the water vapor transmission rate for PBAT-montmorillonite clay composite films by a significant amount (25.21%), and they attributed this increase to the uniform dispersion of the filler (Mondal et al. 2014)

Poly Vinyl Alcohol (PVA)

Satyajit Gupta et al. evaluated the average transmission rates for different time intervals for PVA/ZnO composites. They created reactive barrier films using nano-scale zinc oxide and polyvinyl alcohol (PVA) as the base polymer matrix. They used thermal deposition of calcium on the surface of composite films, and its degradation for studying the barrier properties (Gupta et al. 2012). According to these

investigations, the calcium layer entirely degraded after 3300 sec for PV4, 200 sec for PV1, and 90 sec for PV0 (PV4, PV1 and PV0 are composites containing 4 wt. %, 1 wt. %, and 0 wt. % ZnO, respectively). The removal of calcium layer can be considered as the measure of gas permeability. The time required for the removal of calcium coating increases with ZnO content in the matrix. The longer amount of time needed to degrade the calcium layer demonstrated how the presence of ZnO in the matrix enhanced the composite's barrier properties. The water vapor permeability values of the composites PV0 to PV1 to PV4 decreased gradually. These results confirmed that the relative reduction in the WVTR is due to increase in ZnO loading.

Commendable improvement in barrier properties of PVA/ graphene oxide (GO) composites were achieved through GO's homogeneous dispersion and effective interfacial contact with the PVA matrix (Kim and Macosko 2008). This investigation shows that GO is a useful nanofiller for creating nanocomposite materials with high barrier characteristics that significantly lower gas permeability. By combining highly orientated and aggregated nanoplatelets with polymer matrix, a gas barrier layer can be created that significantly improves the gas barrier properties of polymer films (Guo et al. 2012).

Ren Peng-Gang and colleagues created a PVA-based nanocomposite film with excellent gas barrier characteristics. Magnetic ferro-ferri oxide nanoparticles ($\text{Fe}_3\text{O}_4@\text{GO}$) were placed on GO sheets to prevent them from moving in a direction perpendicular to the film. The significance of magnetic nanofillers in the matrix is that, by applying magnetic field, gas barrier layer should arrange in the form of congregated network. Then, it was examined how the $\text{Fe}_3\text{O}_4@\text{GO}$ sheets in the PVA matrix affected the oxygen permeability. Oxygen permeability of congregated and oriented PVA/ $\text{Fe}_3\text{O}_4@\text{GO}$ composite films at GO loadings of 0.072 vol % and 0.290 vol % were 0.2126×10^{-15} and $0.1757 \times 10^{-15} \text{ cm}^3 \text{ cm cm}^{-2} \text{ s}^{-1} \text{ Pa}^{-1}$, respectively. In comparison to the neat PVA film, they were 99.0% and 99.2% lower. This work offers a quick and adaptable technique for producing high-barrier polymer nanocomposite films (Ren et al. 2017).

Benoit Van der Schueren et al. investigated the gas barrier properties of composite films containing few layer graphene (FLG) in PVA (Van der Schueren et al. 2020). The barrier properties were in agreement with the nonhomogeneous distribution of and specific arrangement of FLG into connected macroscopic network, with the oxygen transmission decreasing up to 60%, from 10.7 for pure PVA to $4.3 \text{ cc m}^{-2} \text{ day}^{-1}$ for series I-0.5% FLG, and close to this, to 4.5 and $4.7 \text{ cc m}^{-2} \text{ day}^{-1}$ for series II-0.3% FLG and series I-0.5% FLG. The composites exhibited slightly greater transmission at increasing FLG concentrations ($4.9\text{--}6.1 \text{ cc m}^{-2} \text{ day}^{-1}$), which also suggested that the network can expand in the plane rather than in the z direction as more FLG are added.

Poly Lactic Acid (PLA)

Goh et al. prepared sandwich – architected PLA-Graphene composite films and barrier properties were evaluated. The PLA-Graphene composite film was bound

with PVP solution. The PLA film has demonstrated good mechanical and physical qualities, but its water and gas molecule barrier properties are limited (Auras et al. 2003; Li et al. 2014). They used pressure-assisted filtration as a pretreatment technique since the produced rGO core barrier, which had a thickness of less than 5 nm, could not withstand surface abrasion. Polyvinylpyrrolidone (PVP), the binder utilized, is safe for human consumption. The synergistic sandwiched architecture provides good barrier properties and imparts mechanical strength to the composite film. The rGO film improves the barrier property, while the PLA films provide the necessary mechanical support. A 55–65 weight percent PVP binder is necessary for strong PLA–rGO adhesion that is free of delamination. To preserve the integrity of the rGO core barrier throughout the fabrication process, they optimized the concentration of the PVP binder to be at 65 weight percentage (Goh et al. 2016).

Anna J. Svagan et al. studied the Oxygen and Water Vapor Permeability through PLA-Montmorillonite/chitosan bilayer composite films (Svagan et al. 2012). OTR and WVTR were measured according to ASTM standard F2622–08 and E398–03, respectively. Examining the PLA films' multilayer coatings' oxygen barrier qualities, it was found that the OP significantly decreased as the number of bilayers increased. To avoid condensation, the relative humidity (RH) was set at 98% in the wet chamber and 10% in the dry chamber. Between 10 and 70, bilayers were present. The average OP value at 70 bilayers and 20% RH was only $0.255 \times 10^{-2} \text{ mL mm m}^{-2} \text{ day}^{-1} \text{ kPa}^{-1}$, which is significantly less than the value for the plain PLA film ($17.68 \times 10^{-2} \text{ mL mm m}^{-2} \text{ day}^{-1} \text{ kPa}^{-1}$). According to Anna J. Svagan et al., these are the lowest OP values for a PLA-/clay-based film that have ever been recorded. These are the lowest OP values for a PLA-/clay-based film. For comparison with clay loadings ranging from 0 to 10 wt%, Jin-Hae Chang et al. investigated the oxygen permeability tests of PLA-organic clay composite films (Chang et al. 2003). They discovered that with C16-MMT, DTA-MMT, and Cloisite 25A, the O_2 permeabilities dropped from 777 to $327 \text{ cc m}^{-2} \text{ day}^{-1}$, $330 \text{ cc m}^{-2} \text{ day}^{-1}$, and $340 \text{ cc m}^{-2} \text{ day}^{-1}$, respectively. All of the hybrids had oxygen permeability values that were less than half of those of the equivalent neat PLA. Higher clay content films appeared to be much more stiff, which reduced the films' ability to transmit gas.

Traditional PLA/clay nanocomposites typically exhibit a maximum 60% reduction in OP at clay addition levels of 5–10 wt% (0% RH, 23–25 °C) (Chowdhury 2008). A 95 and 90% reduction in OP at 20 and 50% RH, respectively, were accomplished by (Svagan et al. 2012) with PLA films coated with 40 bilayers of MMT/chitosan, which had a total clay content that was substantially lower than that needed for PLA/clay nanocomposites that had been prepared traditionally. These multilayer coated films have adequate oxygen barrier characteristics, making them interesting materials. The layer by layer (lbl) approach enables precise nanometer-scale customization of multilayer coatings.

Chitosan, an antibacterial and nontoxic substitute for the cationic polyelectrolytes, was used in the lbl process to create new multilayer-coated PLA films (Claesson and Ninham 1992). Due to a brick-wall structure of clay platelets and the compact layer structure of the adsorbed chitosan, the oxygen barrier qualities

have been successful. The robust molecular connection between MMT and chitosan is mediated by electrostatic forces and hydrogen bonds (Paluszkiwicz et al. 2011), because it binds the clay to the neighboring polymer and prevents oxygen from penetrating the interfacial polymer layers (Hedenqvist et al. 2006).

Christian Aulin et al. studied the Oxygen and Water Vapor Permeability of PLA/nanocellulose films (Aulin et al. 2013). According to ASTM D 3985–06, the oxygen transfer rate (OTR) of the PLA substrates coated by lbl technique was measured. The uncoated PLA film had an oxygen permeability of $151.5 \text{ cm}^3 \text{ m}^{-2} \text{ day}^{-1} \text{ atm}^{-1}$, while the PLA coated with 20 bilayers of polyethylenimine/nano-fibrillated cellulose (PEI/NFC) had an oxygen permeability of $49.3 \text{ cm}^3 \text{ m}^{-2} \text{ day}^{-1} \text{ atm}^{-1}$. When there were 50 deposited layers, oxygen permeability dropped even further, to $9.0 \text{ cm}^3 \text{ m}^{-2} \text{ day}^{-1} \text{ atm}^{-1}$. PLA, which has a water vapor permeability of $4.8 \text{ g m}^{-2} \text{ day}^{-1} \text{ atm}^{-1}$, improved its water vapor permeability by 30% when a 20 bilayer PEI/NFC coating was applied to it. By contrast, a 50-bilayer coating reduced water vapor permeability by around 50%.

Ningning Wang et al. studied the effect of lignin (LG) with and without bonding agent on the gas barrier properties of PLA films (Wang et al. 2021b). The oxygen permeability of the PLA/LG composite films, with or without the compatibilizer, was used to evaluate the barrier qualities of the composite films. The addition of LG limited the oxygen permeability (PO_2) of the neat PLA sheet. By adding 1 phr LG, the PO_2 was reduced by around 50%. The PLA/LG composites used two different compatibilizers – polylactide-graft-glycidyl methacrylate (PLA-g-GMA) and polylactide-graft-poly (ethylene glycol) methyl ether methacrylate (PLA-g-PEGMA), and they each had a different impact on the oxygen barrier properties. The LG content ranged from 1 to 5 phr. In comparison to PLA-g-GMA, PLA-g-PEGMA can improve the gas barrier performance of PLA and LG composites. Comparing both sets of composites with comparable LG content revealed that PLA/PLA-g-PEGMA/LG composites had improved barrier performance.

During the creation of the films, the LG particles served as nucleating agents to create tiny PLA crystals. These crystals enhanced the barrier properties of PLA/LG composite films because of their impermeable nature, which functioned as a barrier to gas diffusion. To date, most reports on how nanoparticles affect polymer crystallinity have focused on spherical particles (Niyom et al. 2019). The impact of the dispersed particles on nanocomposite gas transport properties was studied by Weigelt et al., and Sadhegi et al. (Weigelt et al. 2018; Sadeghi et al. 2018). Since PLA-g-PEGMA crystallizes more readily than PLA-g-GMA, composite films comprising the former demonstrated superior gas barrier characteristics. The amount of crystallinity in PLA/LG composites affects barrier characteristics more than other factors because the crystalline areas operate as hard regions that prevent gas molecules from penetrating and provide a tortuosity effect. PLA-g-PEGMA serves as both a nucleating agent and a compatibilizer between PLA and LG. Thus, in the presence of PLA-g-PEGMA, newly generated PLA crystals can fill the gaps between LG and act as continuous barrier layers.

PLA/LG composite films were prepared by melt-mixing process. The gas barrier properties of PLA/PLA-g-PEGMA/3LG showed superior gas barrier properties

compared to PLA/graphene oxide (Huang et al. 2014; Pinto et al. 2013) and PLA/montmorillonite composite films (Koh et al. 2008). The factors affecting gas transport performance of composite films are molecular structure and film formation conditions. The film formation process includes preparation method, solvent type in the casting and drying stages (Li et al. 2019).

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Structural, Morphological, and Textural Properties of Biopolymers

13

Geethy P. Gopalan and Saithalavi Anas

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Abstract

Biopolymers are polymeric materials which are derived from natural or biological resources. They are the upcoming opponent of synthetic polymers. They are diverse and versatile class of materials and have a wide range of applications in various fields. They have great interest in the scientific community due to their excellent properties such as ease of handling, reliability, biodegradability, etc. They can be used as adsorbents, lubricants, cosmetics, drug delivery, adhesives, etc. There are several widely used natural biopolymers, such as collagen, cellulose, starch, chitosan, chitin, etc., which are covered in this chapter, highlighting the studies on incorporation of nanomaterials, tissue regeneration, biocompatibility, and biodegradation. Additionally, this chapter discusses artificial biopolymer-based nanocomposites including poly(lactic acid)-, polyglycolic acid (PGA)-, and polycaprolactone (PCL)-based nanocomposites. In this regard, there are different types of fillers that have been used for the fabrication of the

G. P. Gopalan (✉) · S. Anas

Advanced Molecular Materials Research Centre, Mahatma Gandhi University, Kottayam, India

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resulting nanocomposites with natural or synthetic polymer matrix. These fillers include titanium dioxide (TiO₂), hydroxyapatite, zinc oxide (ZnO), silver nanoparticles, etc. depending upon the applications. In this chapter, we talk about the structural, morphological, and textural characteristics of these biopolymer-based systems using common characterization methods like scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and X-ray diffraction and transmission electron microscopy (TEM), among others. In terms of biopolymer research and applications, the evolution of extremely sophisticated and integrated processes has endless promise.

Keywords

Biopolymers · Chitosan · Cellulose · Polyglycolide · Polylactic acid · Biodegradability · Morphological

Introduction

Biopolymers are simple macromolecules which are usually obtained from naturally occurring organisms. These polymers are also formed by different chemical, physical, and biodegradable processes. Biopolymers can be categorized as natural or synthetic depending on their source (Hassan et al. 2019; Varma and Gopi 2021). Even though there are several advantages of using these biopolymers for various applications, the main interest towards these biopolymers is due to its biodegradability and increased environmental compatibility. In addition, the use of renewable resources offers an incentive to expand nonrenewable petrochemical supplies.

Natural biopolymers are generated from natural renewable resources and contain repetitive units like polysaccharides, proteins, and so on. They have outstanding characteristics like biocompatibility, nontoxicity, antimicrobial activity, biodegradability, renewability, low cost, light weight, and adsorption properties. Synthetic biopolymers are defined as polymers that are chemically generated from synthetic monomers or altered from natural polymers. The three main synthetic biopolymers are Poly(caprolactone), poly(glycolic acid), and poly(lactic acid). It undergoes natural degradation without remaining any residues which are harmful to the natural environments. In contrast, natural biopolymers are preferred over synthetic polymers because of their more biodegradable property.

Synthetic biopolymers have great attention over the last few years, due to their distinct features over natural biopolymers in terms of stability and flexibility suitable for a wide variety of applications. These biopolymers found significant applications in the medical field, because of their exclusive properties like controlled release, nonimmunogenicity, stability, and clearance from the body, which are suitable for their application in human body. They are now preferred as a source for medical devices and supplies, due to their high stability compared to natural polymers during transport, formulation, and use. Owing to these advantages, these biopolymers are broadly used in gene therapy, implanted devices, regenerative medicine, tissue

engineering, novel drug delivery systems, and in many other products (Thomson et al. 1995; Engelberg and Kohn 1991; Nair and Laurencin 2006).

Proteins are large macromolecules which are made up of a combination of amino acids. They are amphiphilic biopolymers. It can be classified on the basis of plant proteins and animal proteins. They are nontoxic and biocompatible and also have strong affinity to bioactive compounds through various molecular interactions such as hydrogen bonds, hydrophobic interactions, and π - π interactions (Park et al. 2017). Monomeric units of monosaccharides are repeated to form polymeric carbohydrates known as polysaccharides. Through glucosidic linkage, they are covalently joined (Liu et al. 2018). Mainly, there are two categories of polysaccharides: those of a plant or animal origin. Starch, cellulose, etc. are of plant origin, and chitin and chitosan are of animal origin, respectively. Polysaccharides act on membranes and intracellular communication, as adhesives, as emulsifiers, as capsular layers, or as protective barriers around cells (Kaplan 1998).

This chapter discusses the structural, morphological, and textural properties of some important biopolymers. For the structural and morphological investigations of biopolymers, a variety of analytical methods like Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), scanning electron microscopy (SEM), etc. are used. These extremely advanced methods ensure success in the study of biopolymers as well as in a variety of applications. Therefore, this chapter deals with some of the representative examples of the surface and structural properties of biopolymers which are categorized as natural and synthetic biopolymers.

Natural Biopolymers

Natural biopolymers are materials that are usually found in nature. They may be also defined as biological macromolecules, produced by living organisms. They have a lot of benefits like bioactivity, nature remodeling, and the capability to introduce receptor-binding ligands to cells. The main applications of natural biopolymers are in different fields such as in ophthalmology, dentistry, cardiovascular tissue regenerations, orthopedics, etc.

Collagen

Collagen is the primary building block of vertebrates making up about 20–30% of the total body protein of mammals. It is a fibrous protein, and the individual molecules were threadlike structure. They were arranged together to form a continuous framework all through the body (Harkness 1961). It is a rod-shaped molecule with an approximate molecular weight of 300 kDa, with a length and width of about 3000 and 15 Å [6] (Lee et al. 2001). Collagen appeared in the early stages of development in ancient animals like corals, jellyfish, and sea anemones. By using fibroblasts, collagen is synthesized, which generally arises from reticulum cells or

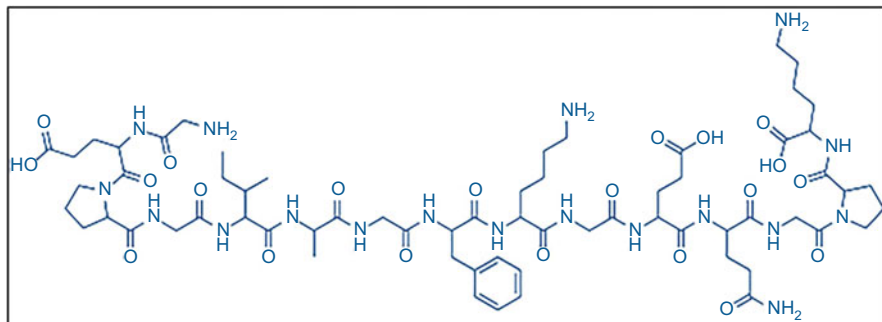


Fig. 1 Chemical structure of collagen

pluripotent adventitial cells. The collagen molecule has a distinctive size, structure, and amino acid sequence. It is made up of three polypeptide chains entwined to form a three-stranded rope (Fig. 1; Teruo and Taira 1992; Rao 1995). There is an individual twist in opposite directions in every chain. The accumulation of amino acid residues and glycine interfered with the development of helical strands. Covalent bonds and hydrogen bonding between adjacent -CO and -NH groups held these strands together. It can be refined into various forms such as gels, sheets, and sponge, and it can be cross-linked with chemicals or undergo physical treatments to change its degradation rate and to strengthen it. The most common structural characteristics of collagens are the presence of a triple-helical structure with a length of 300 nm. The triple helix structure comprises a rodlike structure, which is significant for fibril formation and structural integrity. It also interacts with a broad range of molecules essential in extracellular matrix organization and function (Brodsky and Ramshaw 1997). There are 29 different kinds of collagen in the human body. The range of collagen types is type I through type IV. The protein that is most prevalent in mammals is type I collagen. Three polypeptide subunits of type I collagen exhibited comparable amino acid contents. All polypeptide units had roughly 1050 amino acids, with a proline-, glycine-, and hydroxyproline-to-lysine ratio of about 25%, 33%, and 25%, respectively. Collagen fibrils of types I and V contribute to the structural framework of the bone, whereas those of types II and XI are mostly found in the fibrillar matrix of articular cartilage (David et al. 1988; Richard Mayne 1989; Mark et al. 1984). Mainly two major groups of collagens are present: fibrillar collagen and non-fibrillar collagen. It is classified based on the variations in structure, location, and properties (Ricard-blum 2011; Sorushanova et al. 2018). Fibrillar collagen forms fibrils which constitute around 90% of all collagen presented in the human body. Non-fibrillar collagen is very much different based on structure, properties, and location. Even though it contains only 10% of the collagen in the human body, it is an important part of many organs (Gelse et al. 2003; Mienaltowski and Birk 2014; Holmes et al. 2018). Collagen fibers were connected to form a macrostructure at the nanoscale, as seen in Fig. 2's cartoon representation of the

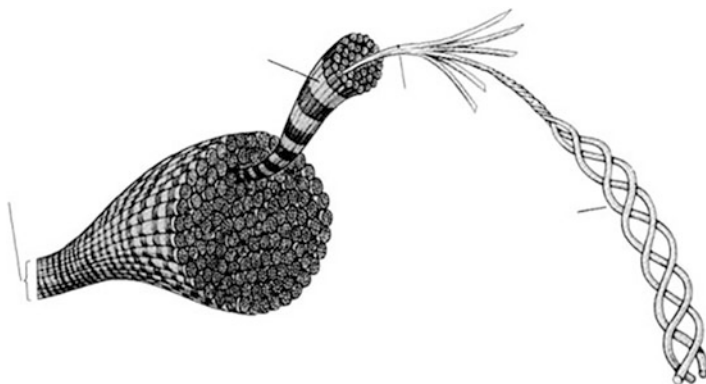


Fig. 2 Cartoon structure of collagen showing the packing (Francis et al. 2014)

collagen fiber structure. Single strands combine to form triple helices, which are then strung together to form fibers and bundles.

According to Astbury and Bell's 1940 theory, the collagen molecule is made up of a single prolonged polypeptide chain with amide groups in the *cis* transformation (Astbury and Bell 1940). They made important progress into the structure of the beta-helix and beta-sheet, while Polling and Cory simultaneously presented a structure for collagen (Linus Pauling 1951). Three polypeptide strands are kept together in a helical conformation by hydrogen bonds in that structure. Two of the three peptide bonds and four of the six main chain heteroatoms involved in the hydrogen bond for each amino acid tripod are required for the development of the structure. Using X-ray diffraction, Ramachandran and Kartha conducted a thorough investigation of the structure of collagen fibers in 1954 (Ramachandran and Kartha 1954, 1955). The size, function, and tissue distribution of various kinds of collagen vary significantly (Gelse et al. 2003).

Collagen-based materials have gained much interest in repairing and replacement of body tissue like heart valves, bones, skin, dentals, vascular grafts, and tendons. There are some general properties that make collagen an interesting biomaterial, such as biocompatibility, hydrophilicity, low antigenicity, its suitability as a substrate for cell growth, high mechanical strength of the fibers, and tunable stability by chemical or physical cross-linking. But it also suffers from some poor physicochemical properties such as mechanical strength, thermostability, and resistance to enzymes. As compared to other biopolymers, collagen is the most important constitutive protein that plays a vital role in the formation of the fundamental building block of connective tissues, and its metabolism correlated with many physiological processes of biological adaptations and tissue regeneration.

There are several works reported based on collagen, like hydrogels, composites, films, etc. Here, Ficaí and his colleagues used a self-assembly approach to create a collagen/hydroxyapatite composite material. The starting components for the *in vitro* mineralization process are collagen and hydroxyapatite precursors. It was shown that by utilizing this method, it was possible to produce composite materials with

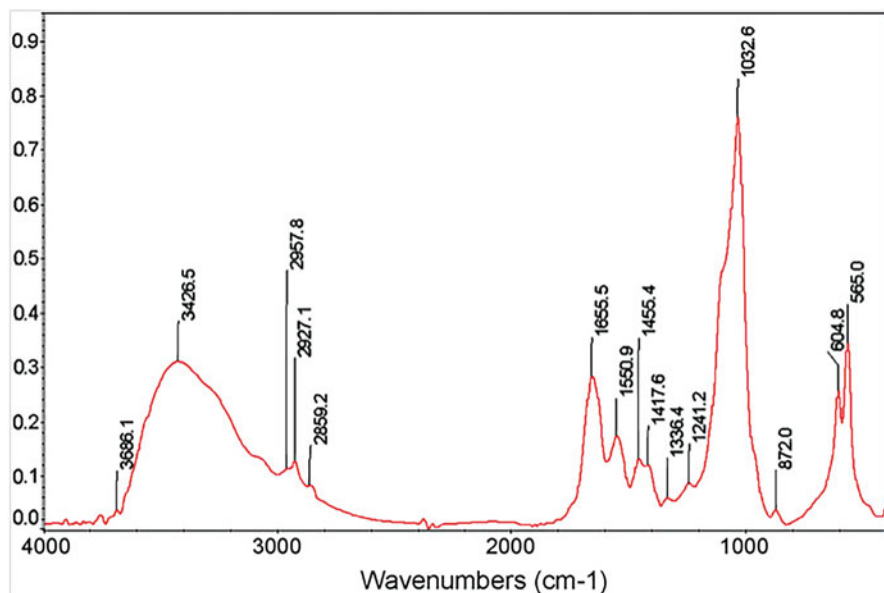


Fig. 3 FTIR spectrum of COLL/HA composite material. (Reproduced with permission from (Ficai et al. 2010))

different morphologies and compositions. The collagen fibers that were produced do not exhibit any orientation (Ficai et al. 2010). The main constituents of the bone tissue are hydroxyapatite and collagen (Sukhodub et al. 2004). The bones acquire their unique mechanical, physical, and chemical properties through the self-assembling process of the organic phase, particularly collagen, the mineral phase hydroxyapatite, and water (Wahl and Czernuszka 2006). The production of these composite materials, which provide structural and chemical similarities to the bone, was the work's primary point of distinction.

FTIR spectrum of COLL/HA composite materials was shown in Fig. 3. The interferogram shows the characteristic peaks of collagen and hydroxyapatite. The characteristic peaks of collagen are obtained in the regions as follows: 1650 cm^{-1} corresponds to amide I, CO , 1550 cm^{-1} which corresponds to NH stretching and C-N bending (amide II); C-N band appears at 1417 cm^{-1} ; and amide III (C-N bending and N-H stretching) vibrations are obtained at 1241 cm^{-1} . The peaks at 2859 cm^{-1} , 2927 cm^{-1} , and 2957 cm^{-1} represent the asymmetric and symmetric stretching vibrations of CH_2 and CH_3 , respectively. The OH stretching vibration is obtained at $3000\text{--}3500\text{ cm}^{-1}$. The main peaks of hydroxyapatite are also shown. There is a peak at 957 cm^{-1} , 565 cm^{-1} , 605 cm^{-1} , and 641 cm^{-1} which corresponds to P-O stretching, O-P-O bend, and triple degenerate. The existence of carbonate group in the composite materials was confirmed by the absorption bands obtained in the region at 1336 cm^{-1} and 872 cm^{-1} corresponding to $(\text{v}_3\text{CO}_3^{2-})$ and $\text{v}_2\text{CO}_3^{2-}$.

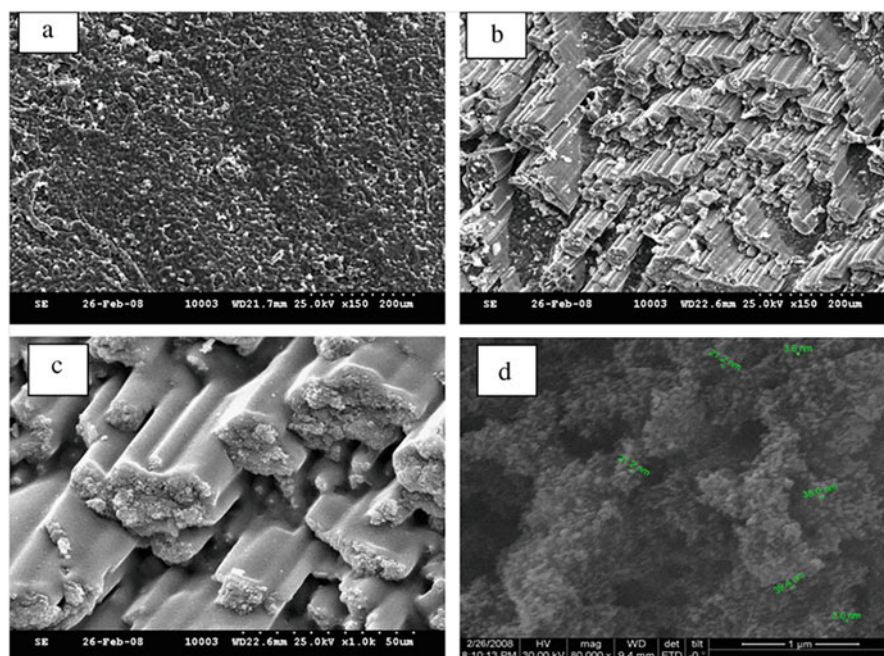


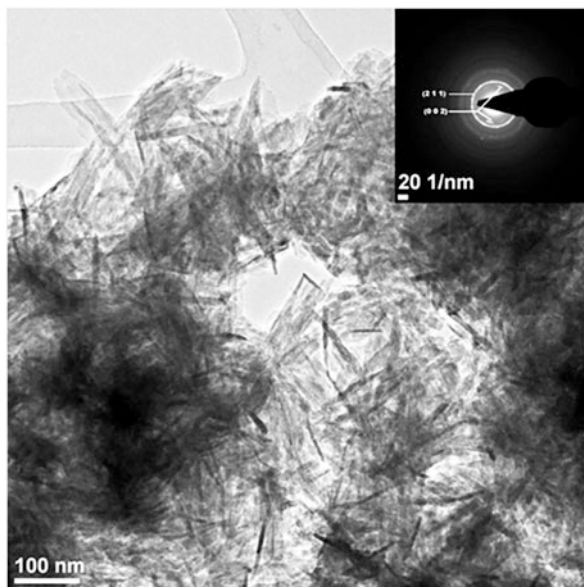
Fig. 4 SEM pictures of collagen/hydroxyapatite composite materials, captured at various magnifications including (a) parallel view with the fibers, (b–c) perpendicular view with the fibers, (d) high-resolution SEM at 80,000 \times magnification. (Reproduced with permission from (Ficai et al. 2010))

SEM images were shown in the figure at different magnifications. Fig. 4 shows a homogeneous arrangement of the fibers. A highly oriented fibers and fiber bundles were observed in the figure. Figure 4b, c images are recorded in fiber's parallel profile. There is a homogeneous arrangement observed in the fiber's parallel profile. While viewing the rupture profile, a shift was observed between the rupture points as compared with the neighboring adjacent layers. Figure 4c clearly depicts the mineral deposition, and the image was viewed at a higher magnification. It is evident that the HA density is higher at the end of the collagen fiber. The heavily mineralized zone of the interfibrillar gap, which is present at the ends of two subsequent fibers, looks to be followed by the rupture profile. When looking at a higher magnification, HA crystals are produced which are in the nanometric range (4–40 nm) and have an enlarged shape.

It should be noted that collagen fibers are destroyed as the sample is prepared, and TEM can only visualize collagen molecules and fibrils (Fig. 5). The collagen molecules and fibrils are attached to hydroxyapatite nanoparticle in an elongated form.

Another major work based on hybrid collagen scaffolds was reported by Kalirajan et al. (2022). They synthesized a novel hybrid collagen scaffold, which

Fig. 5 TEM image of mineralized COLL/HA composite material. (Reproduced with permission from (Ficai et al. 2010))



is prepared by using oxidized inulin (OI) and zirconium dioxide (ZrO_2) nanoparticles. Hybrid biomaterials with metal nanoparticles and active ingredients are becoming more interesting due to their increased wound healing capacity. Here, they hypothesize that oxidized inulin acts as biocompatible cross-linker by interacting with amino groups of collagen molecules. The strength of synthesized collagen scaffolds was increased after the incorporation of ZrO_2 nanoparticles which was used in skin and bone tissue engineering applications. The pore size of the collagen scaffolds is smaller, whereas the hybrid collagen scaffolds show a bigger size, which was confirmed from morphological studies. This is due to the combined effect of the collagen solution's enhanced viscosity following its interaction with oxidized inulin and the presence of ZrO_2 nanoparticles. There is no alteration in the amide I band which is clear from the FTIR spectra. It also reveals that the interaction of ZrO_2 nanoparticles and oxidized inulin did not affect the triple-helical structure of the collagen. This result confirmed that the hybrid scaffolds were obtained with unaltered secondary structure of the collagen.

Collagen-based hydrogels have gained popularity as biomaterials in biomedical engineering over the past few decades as cell or drug transporters, medical implants, and three-dimensional (3D) scaffolds (Mei et al. 2019; Branco et al. 2014; Sun et al. 2017). Zhang and his co-workers described a straightforward method to create a multifunctional hydrogel out of collagen, poly(N-isopropylacrylamide), guar gum, borax, and graphene oxide (GO), by expanding both the reversible and permanent networks (Zhang et al. 2021). The obtained hydrogels (COL-GG-PNIPAMs) display desirable properties, comprising processability, remoldability, and injectability.

From the SEM images, it was confirmed that the synthesized hydrogels exhibit a 3D interconnected porous network structure without any agglomeration of GO particles. The thermal studies demonstrated that the thermal stability also improved for the collagen-based hydrogels.

Cellulose

Cellulose, a polysaccharide and a linear naturally occurring polymer of β -(1 \rightarrow 4)-d glucopyranose, acquires a significant number of hydroxyl groups on its surface, in order to create inter- and intramolecular hydrogen bonds. It is comprised of repeating glucose units $(C_6H_{10}O_5)_n$ (French 2017). It is a key structural element of the principal cell walls found in plants, oomycetes, and various types of algae. The cellulose is mechanically strengthened by the plant cells' cell walls (Khandelwal and Windle 2013). It is able to maintain the semicrystalline state of aggregation while still in aqueous environment, which is rare for polysaccharides. It is a homopolymer of glucose derivative and also an excellent source of fermentable sugar. It is a water-insoluble polymer and also a semicrystalline material. Based on its origin, pre-treatment, and extraction method, the degree of crystallinity was measured. In the cellulose structure, each glucose residue has three hydroxyl groups. The degree of polymerization ranges from 1000 to 15,000 depending on the glucose units. The link is stabilized by intra-hydrogen bonding between the cellulose molecule's hydroxyl groups and the nearby oxygen group. As a result, cellulose chains took on an unbranched shape (Agoda-Tandjawa et al. 2010). These molecules were found in parallel layers organized as primary fibrils (1.5–3.5 nm in diameter) in the tunicate cell walls of algae, plant cell walls, epidermal cell membranes, and bacteria. Van der Waals forces and/or strong intra- and/or intermolecular hydrogen bonds hold the cellulose chains together, giving them a lateral dimension of 3–5 nm. Each elementary fibril is made up of a cluster of cellulose crystals that alternate with irregular domains along the fibril axis. These basic fibrils bundles contain cellulose microfibrils with a cross-sectional width of 5–20 nm and a length of different micrometers, depending on their origin.

Cellulose exists naturally or separated from different sources by some pre-determined shape and dimension. It can be classified into cellulose filaments, cellulose fibers, cellulose micro/nanofibrils, and cellulose crystals. Every cellulosic particle has a specific size, crystallinity, aspect ratio, morphology, and physicochemical properties (Seddiqi et al. 2021). It is the main component of plant fibers enclosed by hemicellulose and lignin. Cellulose chains are organized into fibrils in plant cell walls forming microfibrils of a few nanometers in diameter. Figure 6 shows the ordered arrangement of microfibrils (Joseph et al. 2020).

Cellulose in nanometer size range (10 nm–50 nm) is known as nanocellulose (NC). They have a wide range of applications because of their distinctive properties like low density, biodegradability, and good mechanical properties. Adil et al. reported the isolation of nanocellulose from flaxseed hull by acid hydrolysis. FTIR spectra of cellulose nanocrystals (CNC) were shown in Fig. 7 (Farooq et al. 2020).

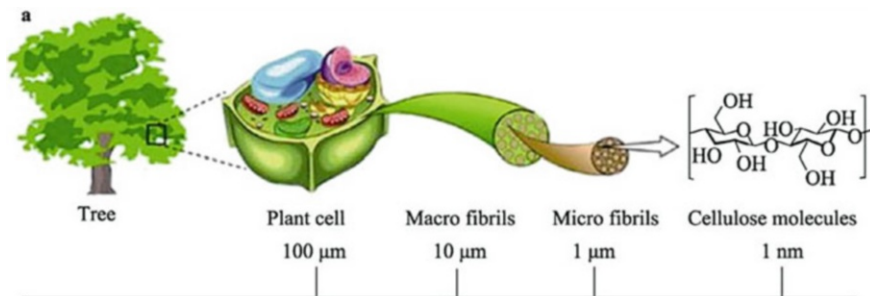


Fig. 6 The hierarchical structure of the cellulose found in plants and trees ranges in size from meters to nanometers, as shown in (a). Reproduced with permission from Multidisciplinary Digital Publishing Institute (MDPI) (Miyashiro et al. 2020)

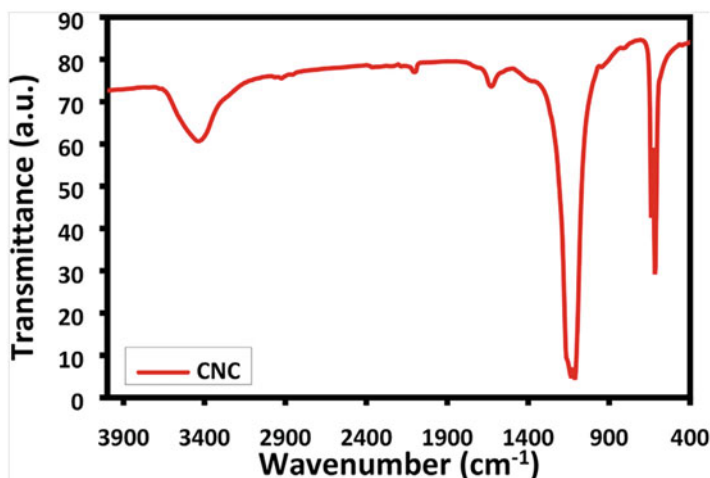


Fig. 7 FTIR spectra of the CNC. (Reproduced with permission (Farooq et al. 2020))

The spectrum reveals that the nanocrystals exhibit a wide band observed around 3400–3500 cm^{-1} , depicting the OH stretching vibrations of cellulose from adsorbed water (Orasugh et al. 2018). The aliphatic saturated (CH_2) stretching vibrations in cellulose and hemicellulose are shown by the peaks at 2920 cm^{-1} . Due to the presence of lignin, another peak was produced in the region at 1627 cm^{-1} that is associated with the C-C vibration. The peak at 1458 cm^{-1} depicts the C-O stretching vibrations, and in addition there is another peak at 1160 cm^{-1} ascribed to the C-O-C stretching of CNC (Rani et al. 2019).

The surface morphology was measured using scanning electron microscopy (SEM) (Fig. 8). CNC acquires a granular surface morphology where individual particles are attached to one another with some agglomerations. Further research using TEM revealed in Fig. 9 that the flaxseed hull fraction-derived CNC featured a

Fig. 8 SEM micrograph of CNC. (Reproduced with permission (Farooq et al. 2020))

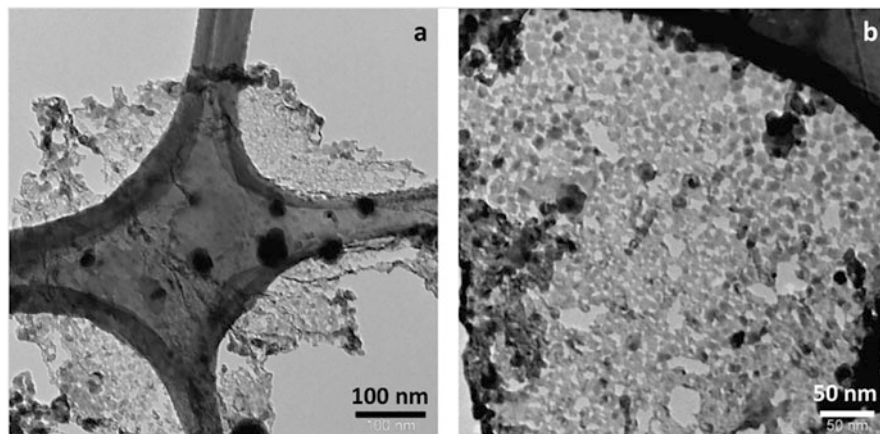
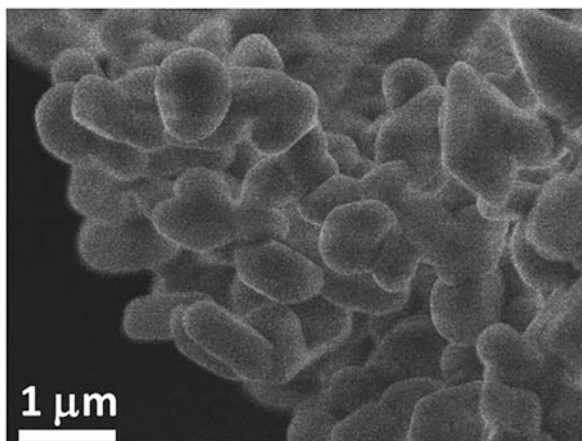


Fig. 9 TEM images of (a) CNC film and (b) granular crystal obtained on CNC films. (Reproduced with permission (Farooq et al. 2020))

film-like surface made up of spherical grains that was visible under magnification. The granules of nanocrystals had an average particle size of 6.28 nm.

The textural and surface morphological evaluations were determined using nitrogen adsorption-desorption isotherms. N_2 adsorption-desorption isotherms were attained and shown in Fig. 10. The figure illustrates that the isotherm is type II b according to the classification by Brunauer and his co-workers. The IUPAC depicts the collection of nonporous granules (Stephen et al. 1940). The pore size distribution of CNC was shown in Fig. 11. The CNC's pores were tiny, and an average pore volume of $0.2 \times 10^{-3} \text{ cm}^3/\text{g}$ was noted.

Natural carbohydrate biopolymers with enough hydrophilic groups include cellulose and compounds generated from it (Sarkar and Singh 2017; Sabzalian et al.

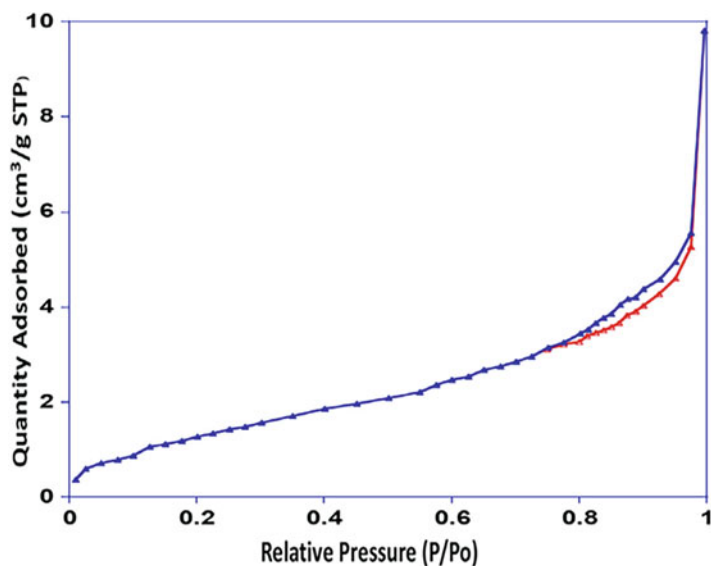


Fig. 10 Nitrogen adsorption-desorption isotherms for the CNC. (Reproduced with permission (Farooq et al. 2020))

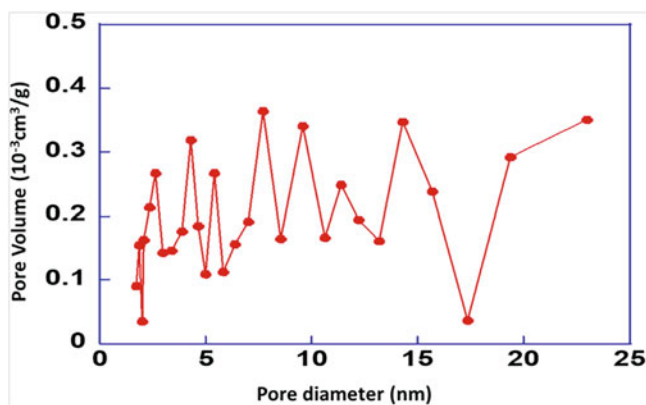


Fig. 11 Pore size distribution of CNC. (Reproduced with permission (Farooq et al. 2020))

2014; Jeong et al. 2018). Owing to its excellent properties such as biodegradability, low cost, and abundant sources, these materials were extensively applied for expanding superabsorbent hydrogels (SHs) (Mignon et al. 2019; Fekete et al. 2014). Sodium carboxymethyl cellulose (CMC-Na) is more soluble in water than cellulose and can create a hydrogel when combined with distilled water. Using sodium carboxymethyl cellulose (CMC-Na), acrylic acid (AA), and 2-acrylamido-2-methylpropanesulfonic acid, Guo and colleagues created a superabsorbent hydrogel (AMPS). In locations with drought and salty soil, it is mostly utilized for soil

conditioning applications that need water absorption and salt tolerance. This work presents a novel process that generated cellulose-SH by exchanging AMPS with CMC-Na. FTIR spectroscopy was used to examine the CMC-Na hydrogel's structural characteristics. The characteristic peaks were obtained after the addition of CMC-Na and AA with different ratios. The results demonstrated that the CMC-Na had been attached onto the polymers. From the morphological studies, grafting of CMC-Na onto polymer chains exhibited a porous anatomy. The size and density of the pores were varying and also irregular. The presence of cavities in the hydrogels confirms the water absorbency capacity. The hydrogel's pore structure became more compact and dense as the concentration of CMC-Na rose and the amount of AMPS reduced. The swelling study also confirms that the CMC-Na graft was successfully grafted onto the structure of hydrogels (Guo et al. 2022).

Starch

Starch is a biopolymer of anhydroglucose units which is linked by $\alpha \rightarrow 4$ linkages and is also a special form of carbohydrate. It is one of nature's energy sources, and it is one of the cheapest biodegradable polymers. This is one of the most abundant naturally occurring biopolymers (Shrestha and Halley 2014). The carbohydrate is stored in various plant parts such as in the roots, pollen seeds, fruits, leaves, cereal grains, tubers, and stem piths. It appears as distinct granules and varies in size and shape based on the botanical source. The physical characteristics, organization, and the chemical composition are also basically common of the biological origin of the starch. It is semicrystalline, water-insoluble, and dense in the granule form. It contains only a small amount of water, so a large amount of carbohydrates is stored in small volume. It is largely extracted from wheat, rice, corn, and potatoes. It is made up of generally two polysaccharides especially amylopectin (100–400,000,000) and amylose (molecular weight of up to 2,000,000) (Fig. 12). The D-glucose units that make up these molecules number in hundreds or thousands. They are linked by α -D-(1 \rightarrow 4) and α -D-(1 \rightarrow 6) glucosidic connections to create linear polymer chains (amylose) and branching chains (amylopectin), respectively. Glucose is the basic building block in both molecules. These molecules may be small amounts of proteins, phosphorus, lipids, and some minerals (Tester et al. 2004). Most plants contained between 70 and 85 percent amylopectin and between 15 and 30 percent amylose. The size of both starch granule molecules, however, can be greatly impacted by mutations that influence starch production.

Amylopectin is the highly branched component of starch, whereas amylose is the linear or barely branched component. Amylose is significantly smaller than amylopectin. The amylose and amylopectin molecules are arranged in the starch granules in a well-ordered manner, which gives the granules their crystallinity. Because of the hydroxyl groups on the granule surface, these starch granules exhibit hydrophilic characteristics and a significant intermolecular contact through hydrogen bonding (Johnson et al. 2003).

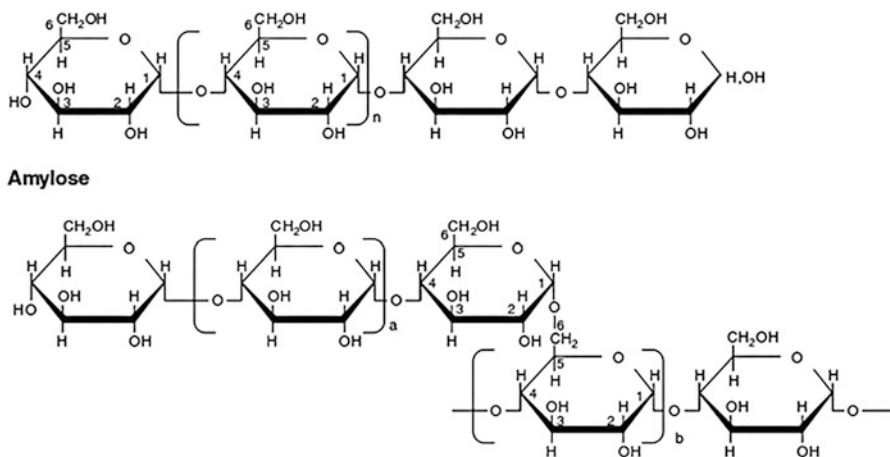


Fig. 12 Structure of amylose and amylopectin

The morphological characteristics of starches derived from various plant sources vary depending on the genotype and cultural practices. The biological origin of starch granules influences their variety in shape and size. It depends on the physiology of the plant and the biochemistry of the chloroplast. Scanning electron microscope (SEM) was used for characterizing the morphological studies. From SEM analysis, it was observed that the granular structures of various plants such as potato, rice, wheat, and corn illustrate major variations in size and shape. SEM images of the starch granules from different plants were shown in Fig. 13. The typical granule size for little potato starch ranges from 1 to 2 mm and from 20 to 110 mm for large potato starch, according to the SEM image. It has been observed that potato starch granules were irregular and oval or cuboidal in shape. The granule size of rice was in the range from 3 to 5 mm, and it appeared as pentagonal- and angular-shaped. The typical granule size and form of maize starch are angular and range from 1 to 7 mm for tiny granules and from 50 to 20 mm for large granules. Two different forms of starch granules – the large A- and little B-type – were found in wheat starch. A-type granules typically range in size from 10 to 35 mm and have a disclike or lenticular form. In type-B starch granules, the size is ranging from 1 to 10 mm in diameter, and they appeared as roughly spherical or polygonal in shape. According to the SEM study, the surfaces of the granules made of rice, wheat, and corn were smoother than those made of potato starch (Singh et al. 2003).

The production of biodegradable, renewable, and environmentally friendly materials has greatly benefited from the use of starch and its nano-derivatives. Starch nanocrystals (SNC) have been extensively used in different fields because of their biodegradability, biocompatibility, availability, low price, renewability, and good biosafety. The diameter of potato starch nanocrystals was about 40–70 nm, pea SNC was about 30–150 nm, and the waxy corn SNC was 50–100 nm (Chen et al. 2008b; Chen et al. 2008a; Yu et al. 2008). Sai Li and his co-workers synthesized

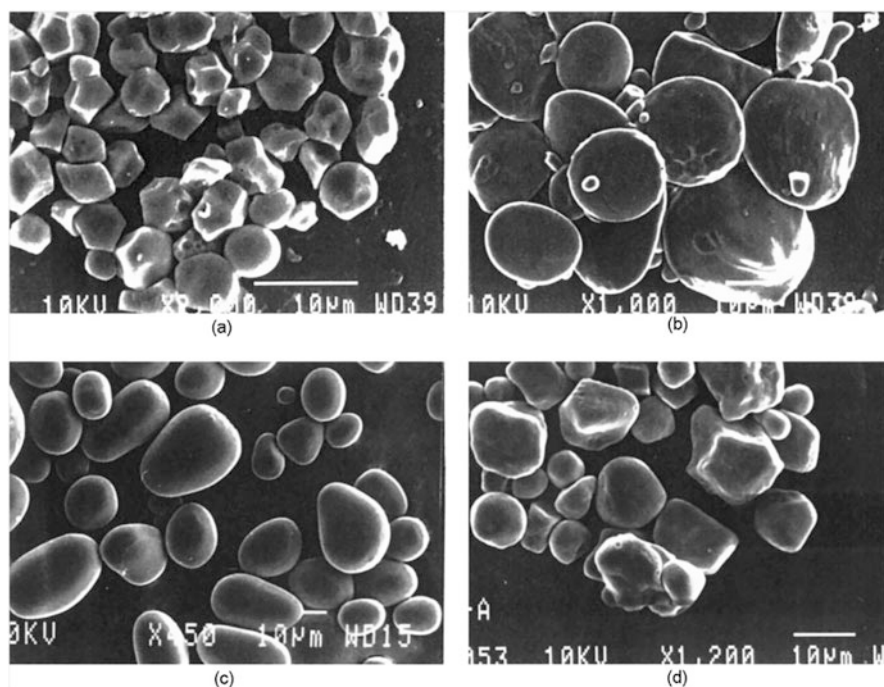


Fig. 13 SEM images of starches from several sources that have been separated (bar = 10 mm): (a) rice, (b) wheat, (c) potato, and (d) corn. (Reproduced with permission from (Singh et al. 2003))

graphene-SNC nanocomposite films which are made by merging reduced graphene (RGO) and starch nanocrystals (SNC). The microstructure and morphology of the composite film were significantly impacted by SNC, which also had a greater influence. Starch's large number of functional groups can offer the GO strong interaction sites for binding. The composite materials with 10% RGO-SNC (front view) were observed as a plain sheet of RGO with a wrinkled structure on the surface based on morphological analyses. SNC was uniformly dispersed in the matrix. The diameter of SNC particles was altered with a small diameter of about tens of nanometers and a large diameter of about 200 nm. The reason was due to the aggregation of some SNC particles. The cross-sectional morphology of 10% RGO-SNC reveals that a small amount of granules between the layers and also a clear and thick layered structure were observed. From this it is confirmed that the SNC is incorporated between the layers of graphene. Using FTIR studies, the characteristic peaks of SNC were observed at 340 cm^{-1} , depicting the O-H stretching vibration. The starch's C-H stretching vibration, O-H bending vibration, and hydroxyl group stretching vibration are represented by the other significant peaks found at 2942, 1640, and 1022 cm^{-1} , respectively. Another peak at 2942 cm^{-1} ascribed to the CH_2 stretching vibration for 10% GO-SNC confirms the successful combination of GO and SNC. This study showed that the inclusion of SNC didn't

prevent GO decrease and that its incorporation enhanced the electrical and mechanical capabilities of the composite membranes.

Chitosan

Chitin has been partially deacetylated to generate the linear polymer known as chitosan. Additionally, it is a copolymer made up of units of β -(1 \rightarrow 4)-2-acetamido-D-glucose and β -(1 \rightarrow 4)-2-amino-D-glucose. It is a crystalline, cationic, hydrophilic biopolymer with exceptional gelation and film-forming capabilities. The deacetylation process is comparatively severe, and it contains the removal of acetyl groups from the chitin molecular chain and the removal of complete amine groups (NH_2) after prolonged treatment with concentrated NaOH. Chitosan was prepared by mainly four major steps from crustacean shells which are (1) demineralization, (2) deproteinization, (3) decoloration, and (4) deacetylation which is demonstrated in Fig. 14a. Chitin includes 5–8 percent (w/v) N_2 , in the form of primary aliphatic amino groups similar to those found in chitosan, depending on the degree of deacetylation (Dutta et al. 2004). Chitosan has the highest metal coordinating ability of all natural polymers in adsorption because it contains reactive primary, secondary, and amino/acetamido functional groups (Prashanth and Tharanathan 2007; Begum et al. 2021). Chitosan is insoluble in the majority of organic solvents and is only soluble in diluted acids such as hydrochloric acid (HCl), acetic acid, and formic acid (Islam et al. 2017; Younes and Rinaudo 2015).

Due to its superior qualities, chitosan-based materials in various forms, such as nanoparticles, nanocomposites, and hydrogels, have been used in biomedical

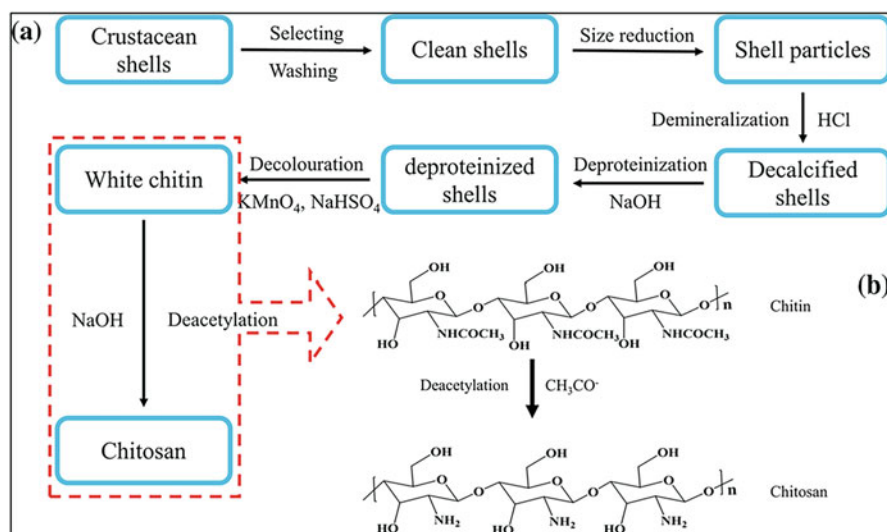


Fig. 14 Schematic for the preparation of chitosan. (Reproduced with permission (Liu et al. 2022))

applications. These attributes include nontoxicity, antifungal, biocompatibility, and anti-tumor activity (Safari et al. 2015; Xue et al. 2015; Ali and Ahmed 2017). It is an outstanding material for medical, cosmetic, and pharmaceutical applications and the preservation of agricultural products (Ibrahim et al. 2020a; Ibrahim et al. 2020b). It is also used in food industry, wastewater treatment, and many other industrial applications (Argu et al. 2006). The major plus point offered by chitosan-based nano-encapsulations is the ability to improve the solubility rate of poorly soluble drugs, thus increasing their bioavailability. This ability relies upon the particle size and specific properties of chitosan, which makes this polymer a suitable drug carrier. One of the major works in the field of composite scaffolds was reported by Olad and his collaborators. The synthesis was carried out using chitosan/gelatin/nano-hydroxyapatite-montmorillonite by means of freeze-drying method for tissue engineering applications. After the mixing of nano-hydroxyapatite and montmorillonite, the swelling behavior of the composite was decreased. The invention of nano-hydroxyapatite and montmorillonite barriers, which controlled the water molecules without allowing them to enter the composite structure, was largely responsible. Additionally, the hydrophilicity of gelatin was reduced by the presence of nano-hydroxyapatite and montmorillonite by joining calcium and phosphate with hydrophilic groups like NH_2 or $-\text{COOH}$ that are present on the gelatin backbone structure (Olad and Azhar 2014).

Menazea et al. reported a new, simple, low-cost, cost-effective antimicrobial composite based on chitosan loaded with different amounts of tigecycline (TIGE) using casting method (Menazea et al. 2020). The US Food and Drug Administration has approved the tetracycline derivative TIGE, which has a low toxicity (FDA) (Bai et al. 2018). They are widely used in antibacterial composites for the treatment of different bacterial infections. The obtained composite materials were characterized by various techniques like FTIR, SEM, etc. FTIR of pure chitosan tigecycline drug and the composite of CS/TIGE in five different ratios were shown in Figs. 15 and 16. A broad peak obtained in the region at 1650 cm^{-1} , 1600 cm^{-1} , and 1640 cm^{-1} corresponds to amide I from chitosan, TIGE, and the composite, respectively. The NH vibration of amide II is obtained at 1560 and 1533 cm^{-1} for CS and TIGE and the composite. The other major peaks of chitosan were at 1405 , 1316 , 1079 and 1038 cm^{-1} depicts the CN stretching, CH_3 (Amide II), C-O-C stretching and CO stretching vibrations. In the composite material, there is a band that disappeared, and other bands were shifted to 1425 , 1086 , and 1018 cm^{-1} in the same arrangement. But the characteristic peak of CS has not been shifted for the composite material. A new peak obtained in the region at 1709 cm^{-1} corresponds to C-O stretching vibrations (Mansour et al. 2017). The reason is because the positively charged amino groups of TIGE and the negatively charged groups of chitosan interact ionically (Faneca et al. 2011).

The morphological studies were carried out using FESEM. SEM images of pure CS, chitosan doped by different concentrations of TIGE such as CS/TIGE1, CS/TIGE3, and CS/TIGE5 samples, was shown in Fig 17 respectively. The pure CS films show a homogeneous nature as already reported in literature (Fig. 17a). In Fig. 17b, at the low doping (CS/TIGE1), the homogeneous film begins to acquire a

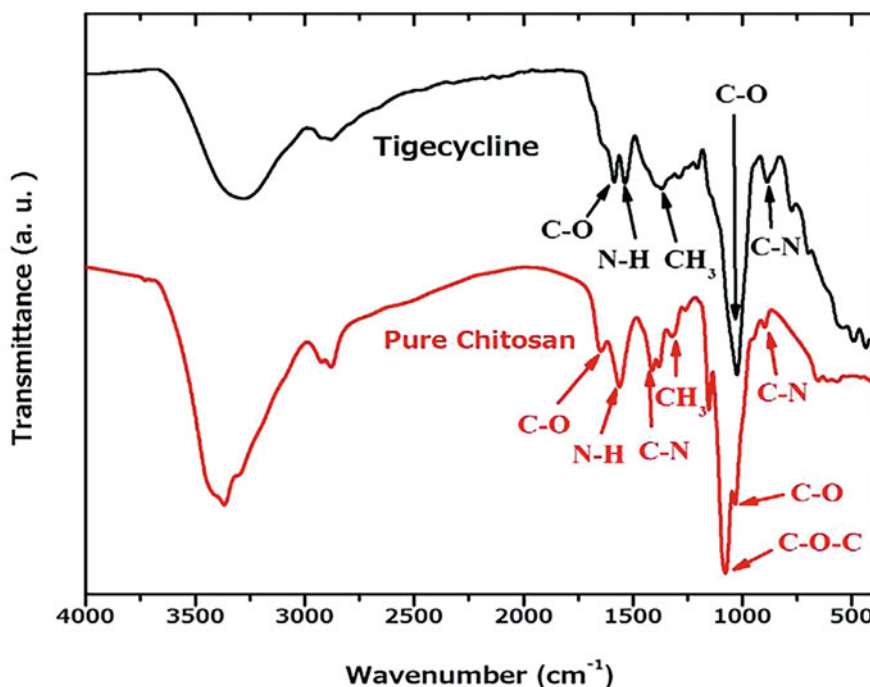


Fig. 15 FTIR spectrum of pure chitosan and TIGE. (Reproduced with permission (Menazea et al. 2020))

small amount of white bright TIGE that is dispersed across the sample's surface. These white brights were increased as the concentration of TIGE was increased which is clearly observed in Fig. 17c. But in Fig. 17d, TIGE concentration was increased; some aggregates were observed due to the agglomeration in certain parts in the samples.

The crystallinity studies and the complexation behavior were recorded using XRD analysis. Figure 18 shows the XRD pattern of pure chitosan and tigecycline at 2θ ranging from 5° to 60° . In the XRD pattern of CS, two distinctive broad hump peaks at 9° and 22° were observed (Mohseni-Bandpi et al. 2015; Choo et al. 2016). And the XRD pattern of TIGE also shows peaks at 7° and a broad hump peak at 21° (Ignjatovic et al. 2010). The XRD pattern of CS doped with different concentrations of TIGE showed that the crystallinity was enhanced after the addition of TIGE (Fig. 19). The intensity of the CS peaks was lowered after the incorporation of TIGE content, which shows the interaction between CS and TIGE.

Chitosan-based hydrogels have received a lot of attention recently for use in biomedical applications, but cross-linking them effectively and safely still poses significant difficulties. Chitosan-based hydrogels were created by Dziadek et al. and cross-linked with 2,3,4-trihydroxybenzaldehyde (THBA). Pectin, rosmarinic acid, and bioactive glass were then added (BG). 2,3,4-Trihydroxybenzaldehyde, a

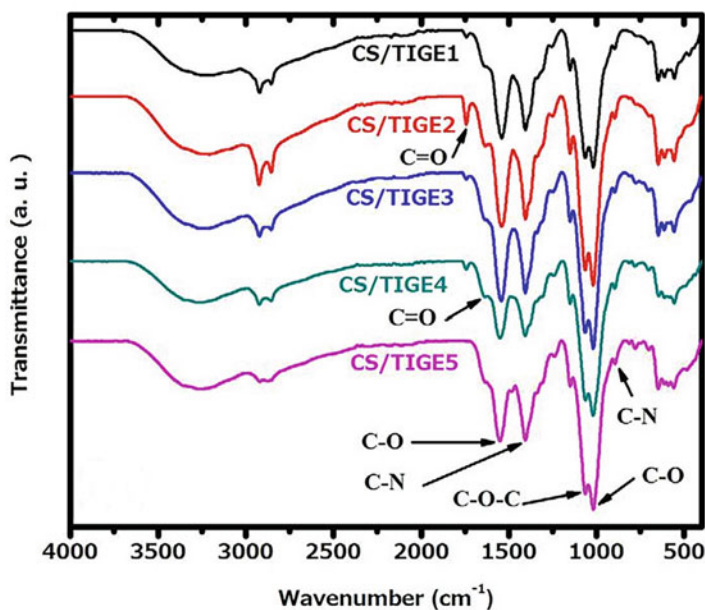


Fig. 16 FTIR spectrum of chitosan doped by different concentrations of TIGE. (Reproduced with permission (Menazea et al. 2020))

phenolic monoaldehyde, was employed for the first time as a cross-linking agent in CS-based hydrogels for potential uses in tissue engineering. THBA acted as a cross-linker, resulting in enhanced mechanical properties, delayed degradation, and high swelling capacity, and it also performed high antioxidant activity and anti-proliferative effect on cancer cells without cytotoxicity for normal cells. The morphological studies reveal that the obtained hydrogels possess irregular highly porous morphology obtained using freeze-drying method. All the materials exhibited sheet- or spongelike structures. As compared to the uncross-linked hydrogels, the pores of cross-linked materials appeared to be smaller. This is due to the lesser amounts of water entrapped between cross-linked CS chains (Dziadek et al. 2022). The hydrogels had pore mostly in the range of 50–150 μm which was confirmed from pore size distribution method and also SEM observations. According to the results, hydrogels are promising multifunctional biomaterials with a wide range of customizable physicochemical and biological properties that have a lot of potential for usage in a variety of tissue engineering fields.

Chitin

Chitin is a natural polysaccharide. It is an abundant biopolymer found in the exoskeleton of crustacean, algae, insect's cuticle, and in the cell wall of fungi. It is

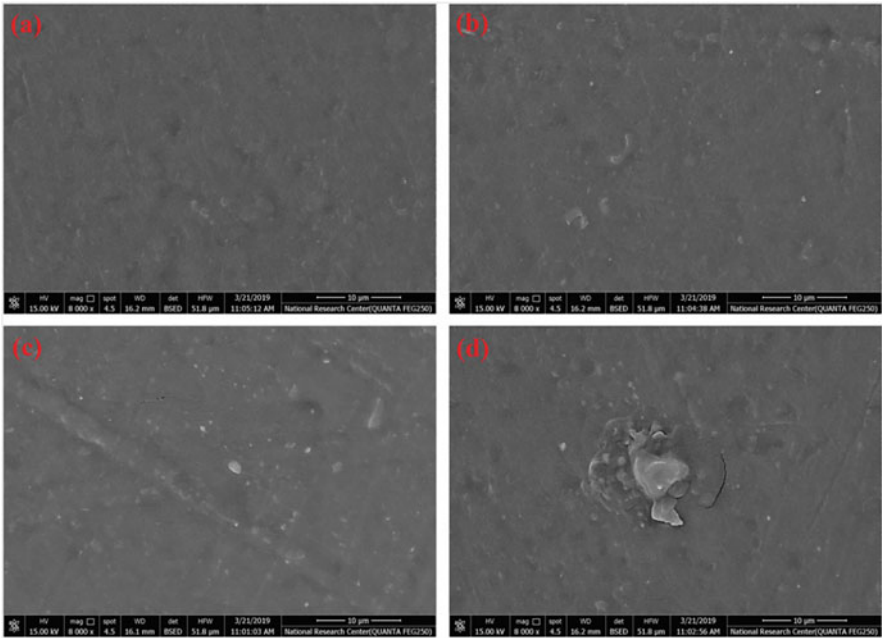


Fig. 17 SEM images of the morphology of (a) pure chitosan and chitosan doped with different concentrations of TIGE, (b) CS/TIGE1, (c) CS/TIGE3, and (d) CS/TIGE5 samples. (Reproduced with permission (Menazea et al. 2020))

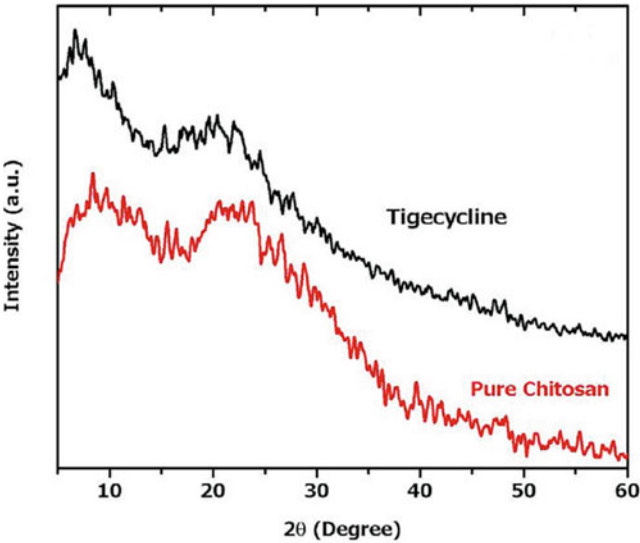


Fig. 18 XRD patterns of TIGE and pure chitosan. (Reproduced with permission (Menazea et al. 2020))

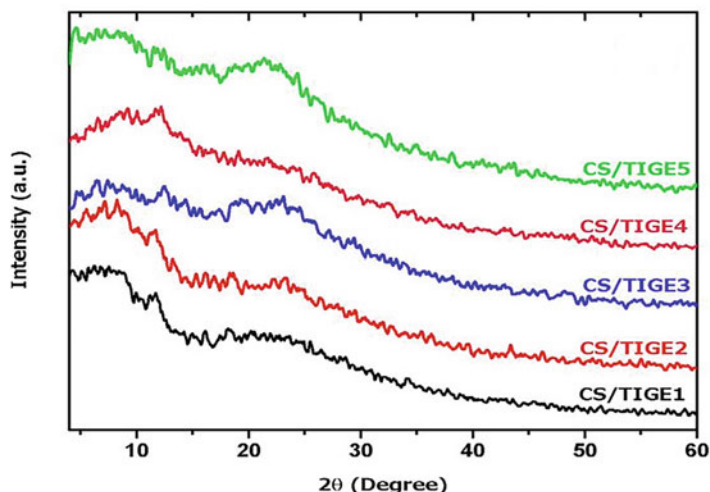


Fig. 19 XRD patterns of chitosan doped with different concentrations of TIGE. (Reproduced with permission (Menazea et al. 2020))

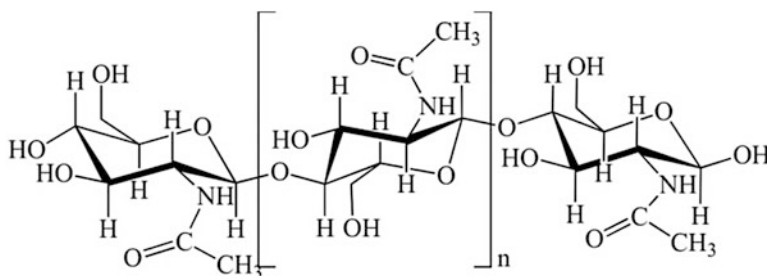


Fig. 20 Structure of chitin

the deacetylated form of chitosan. It is made up of $\beta(1\text{--}4)$ -linked with acetamido-2-deoxy- β -D-glucose. Chitin is nearly identical to cellulose because it has acetamide groups at the (--NHCOCH_3) C-2 locations. The structure of chitin is shown in Fig. 20. The main commercial sources of chitin are shrimp, crab, krill, and lobster. Crustacean chitin is highly connected with minerals, lipids, pigments, and proteins. Industrially chitin extraction involves three steps: (i) deproteinization to remove proteins using aqueous solutions of carbonate, sodium hydroxide, sulfide, sulfite, hydrogen sulfite, potassium hydroxide, carbonate, and calcium hydroxide; (ii) demineralization to remove calcium carbonate using HCl and other acids such as acetic, nitric, sulfuric, and formic acid (No and Hur 1998; Younes and Rinaudo 2015; Percot et al. 2003); and (iii) decolorization to remove pigments with acetone, ethanol, bleaching hydrogen peroxide, etc. (Laurienzo 2010).

Due to its biocompatible, biodegradable, nontoxic, and mucoadhesive qualities, it has been assessed for many applications in the medical, food, chemical, pharmaceutical, and agricultural industries. It is one of the strongest materials for future applications, in competition with petroleum-based polymers. Chitin-based materials have a wide range of applications in various fields. Chitin and its derivatives can be safely applied to both animals and humans. For medical applications, a wide variety of chitin-based products are available, such as porous beads, nonwoven fabrics, finely divided powder, lyophilized soft fleeces or gels, transparent films, laminated sheets, and porous beads (Yang 2011). Kumar and his co-workers synthesized and characterized β -chitin/nanosilver composite scaffolds by using β -chitin hydrogel and silver nanoparticles which are used for wound healing applications. These nano-silver/chitin composite scaffolds have been shown to have antibacterial characteristics after being exposed to *S. aureus* and *E. coli* (Kumar et al. 2010).

Silver molybdate nanoparticles were created in situ in the chitin matrix's microporous structure, which served as a reaction chamber for the creation of $\text{Ag}_2\text{Mo}_2\text{O}_7$ nanoparticles, according to Tang et al. (Tang et al. 2013). CGM networks were made by dissolving chitin in a NaOH/urea aqueous system at -30°C and then cross-linking it with epichlorohydrin. Utilizing a number of different techniques, the produced composite materials were characterized. SEM was used to examine the morphology of the resultant composite materials. Figure 21 shows the SEM image of the $\text{Ag}_2\text{Mo}_2\text{O}_7$ /chitin composite membranes. The CGM displays three-dimensional, porous, stiff, and linked structures. The obtained CGM films had pores with an average size of about 41 nm, which suggests that they had a microporous structure. To form the large pore structure of CGM, the semi-stiff molecular chitin chain significantly contributes to strengthening the pore wall in the gel membrane. In other words, the chitin chains' rigidity supported the hole's wall, allowing it to

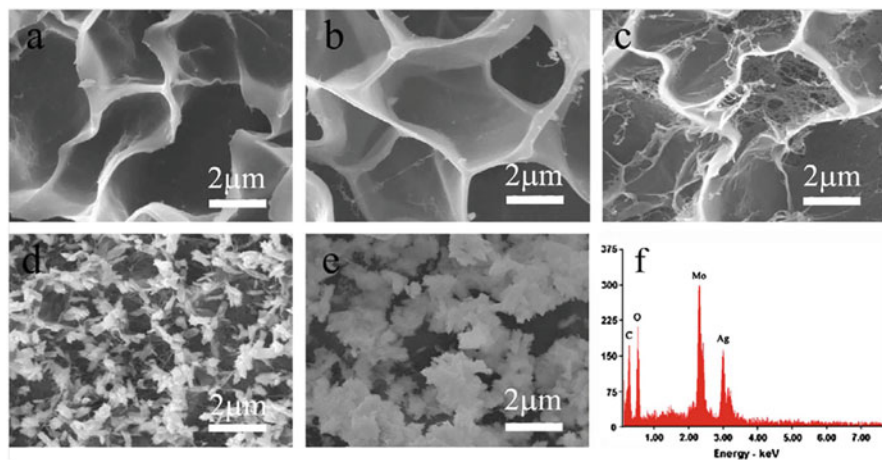


Fig. 21 SEM images of the CGM (a), Ag001 (b), Ag005 (c), Ag01 (d), Ag02 (e), and EDS spectrum (f) from SEM. (Reproduced with permission (Tang et al. 2013))

absorb more water and produce huge pores. As the CGM dried, the size of the pores in the gels rapidly shrank as the heavy water was expelled from the pores (Liu and Zhang 2009). As a result, it is simple to create and immobilize $\text{Ag}_2\text{Mo}_2\text{O}_7$ nanoparticles in chitin matrices. The micropores found in the chitin membrane were regular and had a thin wall, as shown in Fig. 21a. $\text{Ag}_2\text{Mo}_2\text{O}_7$ nanoparticles were filled in the pores of chitin matrices, for the composite membranes (Fig. 21b–e). As the concentration of AgNO_3 increases from 0.001 to 0.02 mol L⁻¹, more $\text{Ag}_2\text{Mo}_2\text{O}_7$ nanoparticles were synthesized, and the $\text{Ag}_2\text{Mo}_2\text{O}_7$ was formed as thicker nanofibers (Kato 2010).

EDS was an analytical method for determining the chemical composition of solid materials. EDS spectrum (Fig. 21f) of the composite membranes was obtained via FESEM. It demonstrated the coexistence of C, O, Ag, and Mo components in the composite membrane, confirming the existence of $\text{Ag}_2\text{Mo}_2\text{O}_7$. As a result, the porous chitin matrix was used as a platform for the in situ synthesis of the nanocomposite membranes.

FTIR spectrum of the CGM and AgO_2 composite membranes were demonstrated in Fig. 22. Chitin's major peaks can be seen at 3430 cm⁻¹, 3270 cm⁻¹, 1660 cm⁻¹, 1623 cm⁻¹, and 1557 cm⁻¹, which correspond to N-H bending vibrations, O-H stretching vibrations, and N-H stretching vibrations (amide I, amide II) (Dong and Ozaki 1997). The hydroxyl and acetyl groups in the AgO_2 composite membrane change typically, from 3430 and 1660 cm⁻¹ to 3420 and 1650 cm⁻¹, respectively. The stretching vibration of the -NH group, which is represented by a minor peak at 3270 cm⁻¹, is lost in the spectrum of AgO_2 due to the strong interaction between the

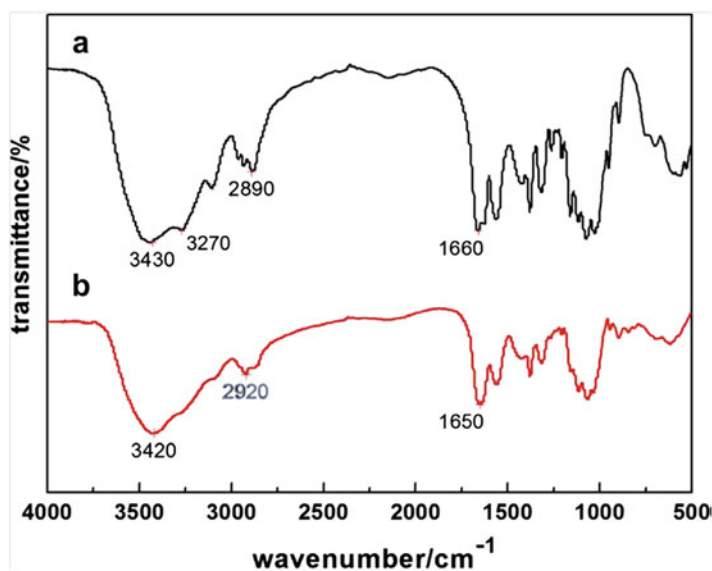


Fig. 22 FTIR spectra of CGM (a) and AgO_2 composite membrane (b). (Reproduced with permission (Tang et al. 2013))

-OH groups and the acetyl groups of chitin in $\text{Ag}_2\text{Mo}_2\text{O}_7$. The chitin backbone's CH stretching vibration is represented by a band at 2890 cm^{-1} , which shifts from 2890 to 2920 cm^{-1} in a significant way. This is another evidence for interactions between chitin and $\text{Ag}_2\text{Mo}_2\text{O}_7$ nanoparticles through hydrogen bonding.

Chitin nanowhiskers (ChWs) Along with other different nanoscale natural filler materials, chitin nanowhiskers (ChWs) have been of tremendous interest for materials applications. Peng et al. successfully fabricated a highly porous, biodegradable, and elastic PVA/ChW hydrogels by physical and chemical cross-linking using glutaraldehyde (GA) for controlled release. The usual peaks of ChWs and PVA are clearly visible in the created composite films' FTIR spectra. No peaks or no changes appeared suggesting that, during the formation of gels, chitin nanowhiskers were physically blended with PVA. Both the materials maintained their own unique chemical structure. SEM micrographs of the hydrogels were carried out. The hydrogel was made using various ChW concentrations. The range of the pore size is from 10 to 100 nm . The pore size was large due to the low ChW concentration. When ChW concentration was increased, an improved microstructure with filamentous structures and smaller pores were observed. As the weight content of ChWs was around 40% , a uniform network structure was examined (Peng et al. 2019).

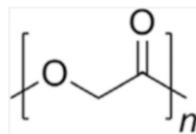
Synthetic Biopolymers

Synthetic biopolymers are either altered from natural polymers or completely synthesized from synthetic monomers. The superior biodegradability and environmental safety of synthetic biopolymers, on the other hand, make them the preferred choice over synthetic polymers.

Poly(Glycolide) PGA or Poly(Glycolic Acid)

Poly(glycolic acid) PGA or poly(glycolide) (Fig. 23) is the simplest and linear aliphatic polyester. It is one of the important synthetic biodegradable polyesters. It has a high melting point (220 – $225\text{ }^\circ\text{C}$) and also shows a higher crystalline behavior. Due to its increased crystallinity, it is soluble in hexafluoroisopropanol (HFIP), up to a molar mass of $45,000\text{ g/mol}$, but insoluble in other solvents (Middleton and Tipton 2000). Its glass-transition temperature (T_g) is in the range of 35 – $40\text{ }^\circ\text{C}$. Since the 1960s, Davis and Geck have used PGA to create the first totally synthetic absorbent suture, known as Dexon (Frazza and Schmitt 1971). Due to PGA's unstable and

Fig. 23 Chemical structure of PGA



easily degradable nature, its industrial manufacturing has proven to be quite difficult, especially when it comes to achieving larger molecular weights (Lu et al. 2014). Although general processing techniques like injection, extrusion, and compression molding can be used to produce PGA in several forms, its high sensitivity to hydrolytic degradations needs a careful control of processing conditions. The monomers used for the PGA synthesis are glycolic acid and glycolide. Ring-opening polymerization of glycolide, polycondensation of glycolic acid, and solid-state polycondensation of halogenoacetates are the three processes used to make PGA. The high molecular weight of PGA is produced by ring-opening polymerization of glycolide (cyclic dimer form of glycolic acid), while the low molecular weight PGA was produced using polycondensation techniques (Dobrzyński et al. 1999; Nieuwenhuis 1992). For industrial applications, it has been synthesized via ring-opening polymerization of glycolide. To initiate the polymerization, Tin octanoate/benzyl alcohol is used as the catalyst system. It can be achieved cationically from glycolide. At room temperature, triflic acid (Lewis acid catalyst) initiates the polymerization reaction (Chamberlain et al. 2000).

In addition to having increased strength and modulus, PGA-derived fibers are also too stiff to be employed as sutures and instead act as tangled material. PGA sutures lose 50% of their strength after two weeks and 100% after four weeks and are entirely dissolved after four to six months. The glycolide monomer was copolymerized with additional monomers to minimize the stiffness attribute.

Zhang et al. synthesized poly(glycolic acid) grafted with chitosan films to enhance the degradable properties of chitosan. The reaction was carried out without using a catalyst. The obtained grafted films were characterized by using various techniques. The variation in glucosamine unit-to-glycolic acid feeding ratios was changed from 1/1 to 1/10. In Fig. 24, IR spectra of chitosan and films with polyglycolic acid-grafted chitosan copolymer that were extracted using methanol were displayed. The largest peaks of chitosan are seen at 1595 cm^{-1} and 1658 cm^{-1} , which correspond to the secondary amide's amide I band and the non-acylated NH_2 group's N-H bending vibrations, respectively. The NH_2 groups of chitosan units are acylated by glycolic acid, and a new peak was identified at 1639 cm^{-1} and 1087 cm^{-1} with regard to the carbonyl stretching of amide and the C-O stretching of glycolic acid $\text{CH}_2\text{-OH}$, respectively, for the PGA chitosan films. The glucosamine-to-glycolic acid molar ratios were raised to 1/5 and 1/10, and a new peak was found at 1749 cm^{-1} and 1184 cm^{-1} that shows ester C=O stretching and oligo(glycolic acid) C-O stretching, respectively. This showed the formation of oligo (glycolic acid) related to chitosan. Moreover, in the spectrum of PGA chitosan, there is no ether peaks between hydroxyl groups depicted, indicating the successful synthesis of poly(glycolic acid)-grafted chitosan.

The morphology of poly(glycolic acid)-grafted chitosan after 4 weeks of degradation was performed using SEM (Fig. 25). Before degradation, the surface of grafted chitosan films and chitosan was found to be smooth. Chitosan film degraded in PBS containing lysozyme and PGA-CS film under the same conditions also showed a rough surface. These findings indicate that PGA-CS film degrades more

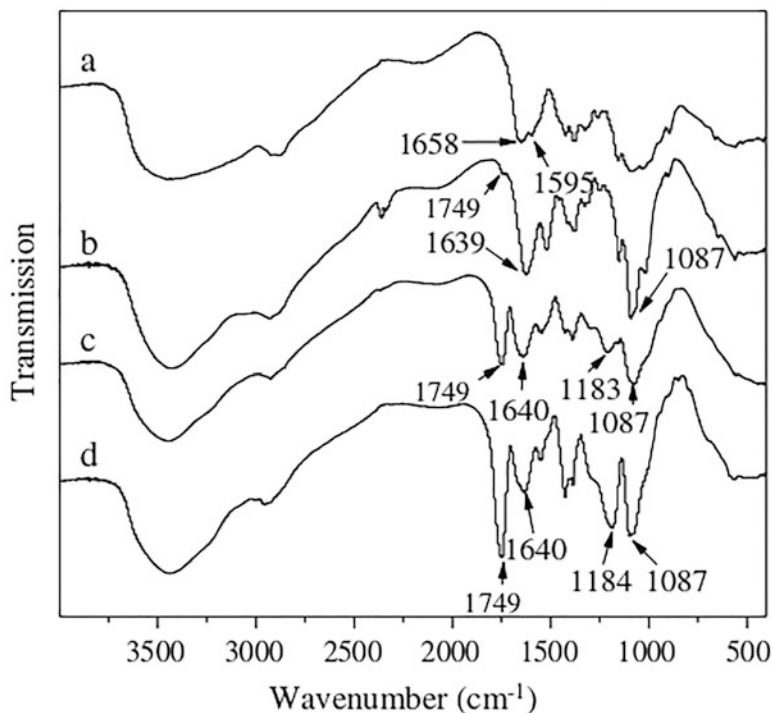


Fig. 24 The feeding molar ratio of glucosamine unit (CS) to glycolic acid is shown in the FTIR spectra of chitosan (a) and the poly(glycolic acid)-grafted chitosan (b, CS/GA = 1:1; c, CS/GA = 1:5; d, CS/GA = 1:10). (Reproduced with permission (Zhang et al. 2013))

quickly than chitosan film, which is consistent with the average weight loss of chitosan that has been grafted with poly(glycolic acid).

The grafted poly(glycolic acid) chitosan film degraded noticeably more quickly than the chitosan film. The breakdown rate of PGA-CS films gradually increased as the molar ratio of glycolic acid to chitosan increased. These findings suggested that chitosan with poly(glycolic acid) grafts might be an excellent biocompatible and biodegradable material.

Poly(Lactic Acid) (PLA)

Poly(lactic acid) (PLA) is both a thermoplastic and a biodegradable polymer. Lactic acid monomers or cyclic dimers are polymerized to make it synthetically. The chemical structure of PLA is shown in Fig. 26. It is created through the fermentation of natural carbohydrates, such as waste from the food or agricultural industries or grains like wheat or maize. It has numerous uses in the delivery of drugs, sutures, stents, and dialysis medium. The cyclic dimer of lactic acid is lactide. Two optical

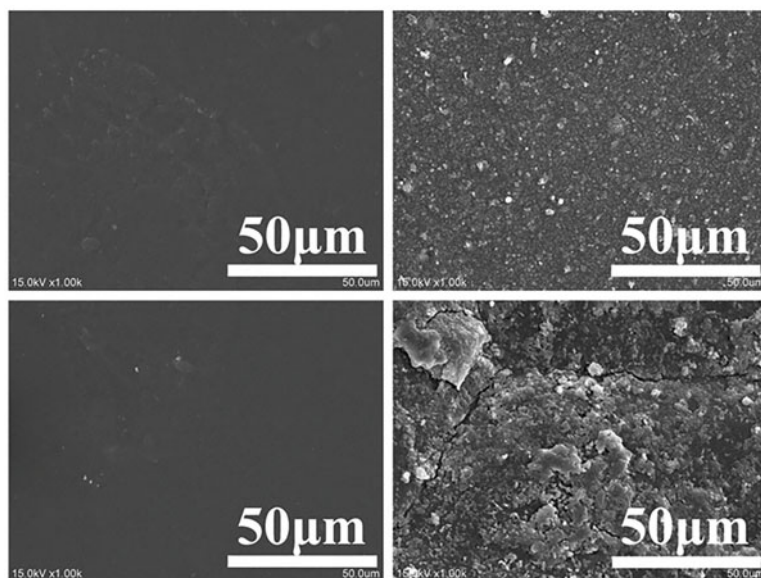
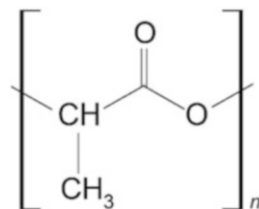


Fig. 25 Chitosan and poly(glycolic acid)-grafted chitosan films were photographed using a scanning electron microscope (SEM) before and after 4 weeks of degradation (**a**, chitosan; **b**, poly(glycolic acid)-grafted chitosan; **c**, chitosan). The glucosamine unit-to-glycolic acid molar ratio in chitosan-g-poly(glycolic acid) is 1:10. (Reproduced with permission (Zhang et al. 2013))

Fig. 26 Structure of polylactic acid (PLA)



isomers, such as D-lactide and L-lactide, are present. The isomer that occurs naturally is called L-lactide. To create DL-Lactide, D-lactide and L-lactide are synthetically combined. The polymerization procedure is similar to glycolide. PLA has a glass-transition temperature of 60–65 °C and a melting point of 175–178 °C and also 37% crystalline (Daniels et al. 1990). PLA was certified by the US Food and Drug Administration as early as the 1970s, and it has been broadly used in clips, plates, screws, sutures, and drug delivery and food packaging applications. The environment to which PLA is exposed determines how biodegradable it is. According to theory, PLA is first hydrolyzed into a polymer, which is then broken down, and the resulting soluble oligomers are then broken down by cells. When removed from the environment, it hydrolyzes into low molecular weight oligomers and mineralizes the microorganisms contained in the environment in CO₂ and H₂O.

PLA has some drawbacks for food packaging applications because of its weak thermal stability, low toughness, ductility, high rigidity, low gas, and water vapor barrier properties (Harada et al. 2007). In order to improve film qualities with additional functional properties like UV-light screening and antibacterial capabilities, PLA is combined with nanofillers such as nanocellulose, nanoclay, and nanometals to overcome these shortcomings (Therias et al. 2012). Along with metal oxide nanofillers, zinc oxide nanoparticles (ZnO NPs) are very remarkable because they achieve many significant properties such as low cost, availability, nontoxicity, stability, strong antimicrobial activity, and high ultraviolet absorption capacity (Shankar et al. 2015). Shankar et al. developed PLA/ZnO NP composite films synthesized by solution casting method. On structural, thermal water barrier, and antibacterial properties, the impact of ZnO NP concentration was investigated. The morphological studies were carried out using SEM. SEM image of ZnO nanoparticles were shown in Fig. 27. It proved that the particles were cubic in shape and ranged in size from 50 to 100 nm. Using ImageJ software, the average diameter of the NPs was determined to be 56.1 ± 18.6 nm. The resulting films had a flat surface and were uniform, transparent, and flexible. Figure 28 shows the FESEM images of PLA and PLA/ZnO NP composite films. While PLA/ZnO NP films reveal a slightly rough surface with nanoparticles scattered in the polymer matrix, neat PLA showed a smooth and compact surface. With higher ZnO NP concentrations, the produced films were more uneven. ZnO NPs developed on the surface of the film when the ZnO NPs were distributed in the chloroform solution in which PLA was dissolved without any surface treatment, as seen by the high concentrations of ZnO NPs.

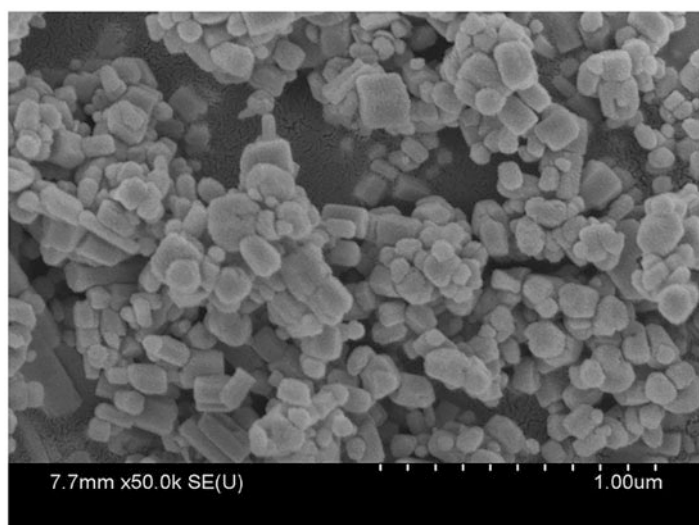


Fig. 27 SEM image of ZnO NPs. (Reproduced with permission (Shankar et al. 2018))

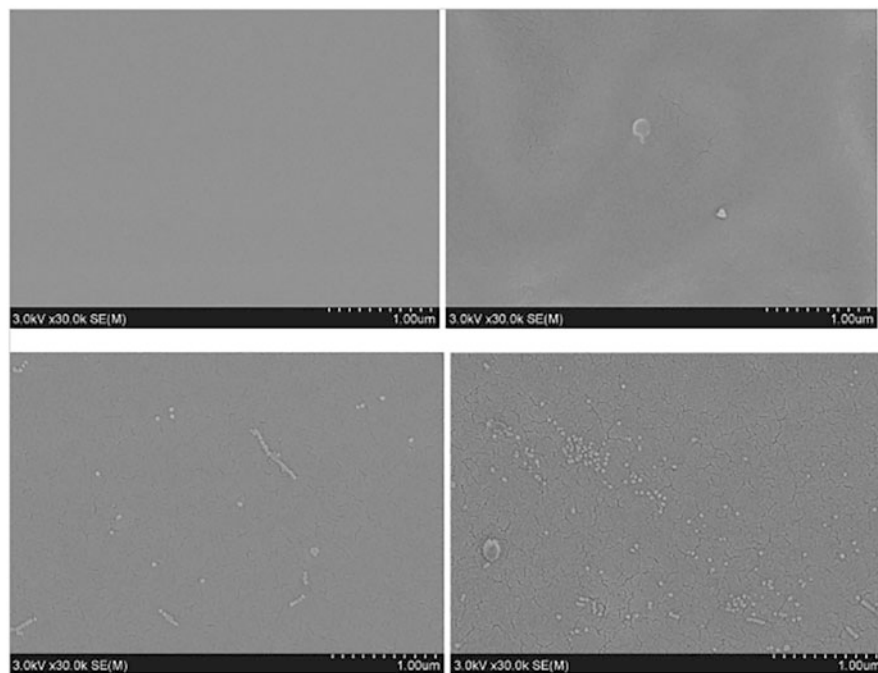


Fig. 28 SEM pictures of PLA and PLA/ZnO NP composite films. (Reproduced with permission (Shankar et al. 2018))

The FTIR analysis was carried out to check the interactions between PLA and ZnO NPs, and the spectrum was presented in Fig. 29. The main PLA peaks, which were obtained at 3658 cm^{-1} , 2931 cm^{-1} , and 1750 cm^{-1} , respectively, show vibrations of the O-H stretching, CH_3 stretching of saturated hydrocarbons, and ester $\text{C}=\text{O}$ stretching in the PLA molecules. The asymmetric and symmetric CH_3 deformation vibration is shown by the peaks observed between 1456 and 1365 cm^{-1} . The PLA's amorphous phase and crystal phase are represented by the other peaks at 871 cm^{-1} and 756 cm^{-1} , respectively. None of the peaks from PLA changed except for an increase in the intensity of the peaks at 3654 cm^{-1} after the addition of ZnO NPs. This finding indicates that the only primary forces present were weak hydrogen bonds between the PLA matrix and the ZnO NPs.

Figure 30 shows the XRD patterns of the PLA-based films. No specific diffraction peak is observed in the range of $30\text{--}80^\circ$ for neat PLA. Clear diffraction peaks were seen in PLA/ZnO NP composite films in the $30^\circ\text{--}40^\circ$ range, which matches with the typical peaks of ZnO NPs (100), (002), and (101). Additionally, Virovska and his colleagues discovered that when nano ZnO was added to the PLA, the distinctive peaks of ZnO were identified at 31.8° , 34.5° , and 36.4° , which correspond to crystal planes 100), (002), and (101) (Virovska et al. 2016). The findings showed that, owing to their antibacterial and UV-light barrier capabilities, the produced PLA/ZnO

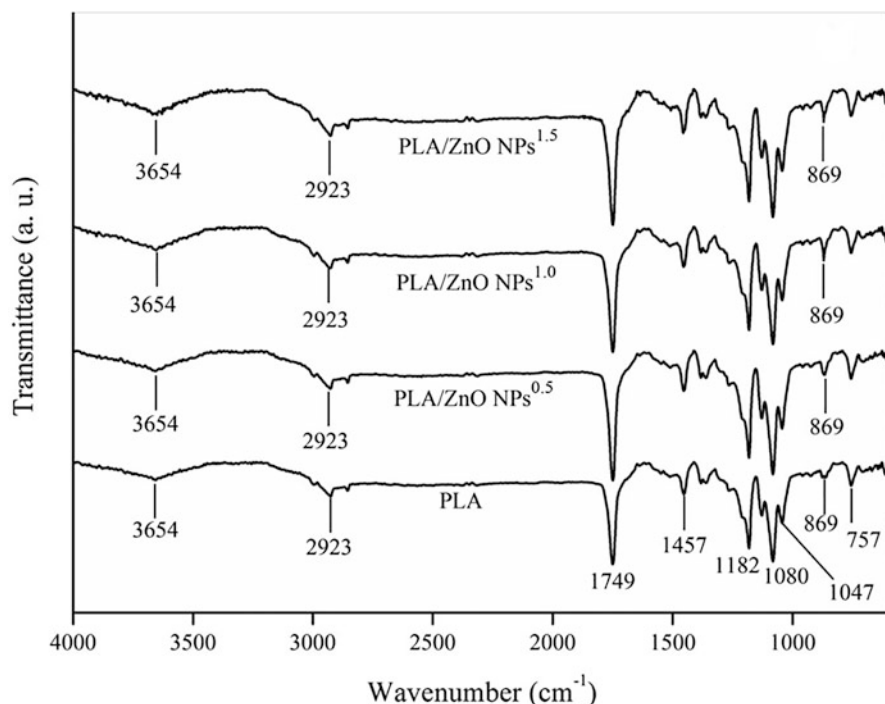


Fig. 29 FTIR of PLA and PLA/ZnO NP composite films. (Reproduced with permission(Shankar et al. 2018))

NP composite films have a significant potential for usage as functional active food packaging and biomedical applications.

Polycaprolactone (PCL)

Polycaprolactone (PCL) is a biodegradable aliphatic polyester. It is a semicrystalline polymer with a glass-transition temperature of -60°C and a melting temperature between 59 and 64°C . Therefore, due to its high toughness and excellent mechanical qualities including elasticity due to its high molecular weight and strength, semicrystalline PCL transforms into a rubbery state at physiological temperature (Bez-wada et al. 1995). It has been of great interest in various scientific and industrial fields since the 1970–1980s. This polyester is unique by its excellent biodegradability, biocompatibility, and mechanical and physicochemical properties. It can be produced through ring-opening and polycondensation polymerization techniques. PCL moiety is hydrophobic, and it is able to form aggregates by its self-assembly behavior after merging with a hydrophilic entity. These aggregates can create sophisticated architectures of extensive biomedical applications. It is widely utilized as scaffolds and resorbable sutures in regenerative medicine and in drug delivery

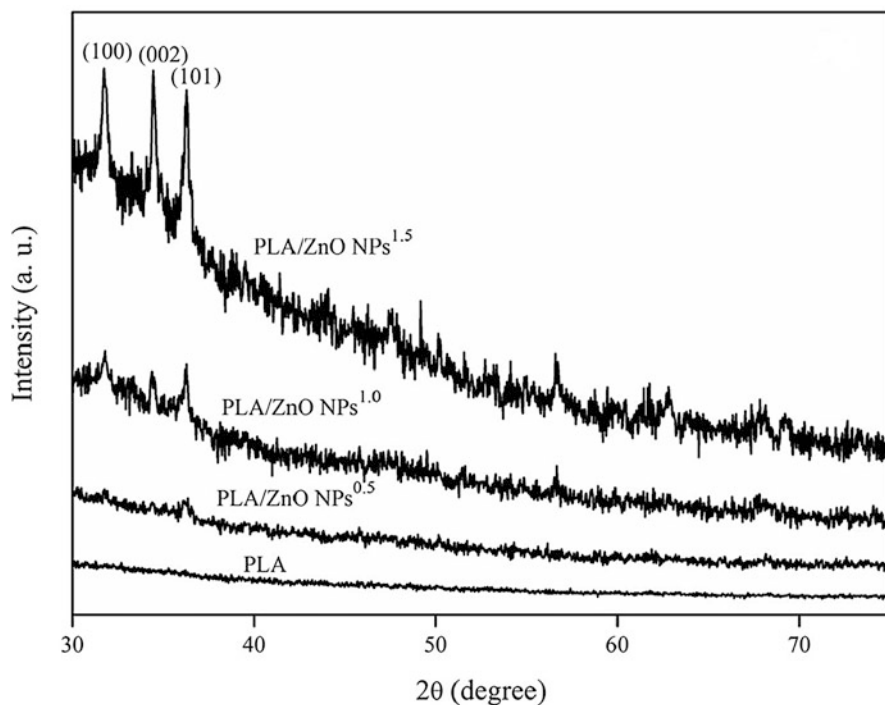


Fig. 30 XRD spectra of PLA and PLA/ZnO NP composite films. (Reproduced with permission (Shankar et al. 2018))

since it is tissue-friendly and nontoxic. PCL degrades more slowly (2–3 years) and is hydrolyzed by either microbes or its aliphatic ester linkage. It is soluble in various solvents like chloroform, tetrahydrofuran, dichloromethane, toluene, cyclohexanone, benzene, carbon tetrachloride, etc. It is less soluble in ethyl acetate, acetonitrile, dimethylformamide, and acetone and insoluble in alcohol and ether (Hedrick et al. 2006). By blending and copolymerization, the physical, chemical, and mechanical properties of PCL can be effectively changed (Azimi et al. 2014). It has been found that after blending, physical and mechanical properties were altered along with biodegradation. For example, mixing of PCL with PLA reduces the Young's modulus and tensile strength; but in the case of PCL/cellulose composites, the crystallinity was increased. Moreover, the copolymerization alters chemical properties, which indirectly affect other properties like crystallinity, degradation, and solubility.

The impact of blending with PLA/PCL and the presence of TiO₂ nanoparticles in the polymers were both examined by Mofokeng et al. While PLA blends with PCL, the overall thermal stability was reduced. By adding TiO₂ nanoparticles, thermal stability was increased. As a result of the incompatibility of PLA domains with weak mechanical properties with PCL, several studies based on the blending of the two

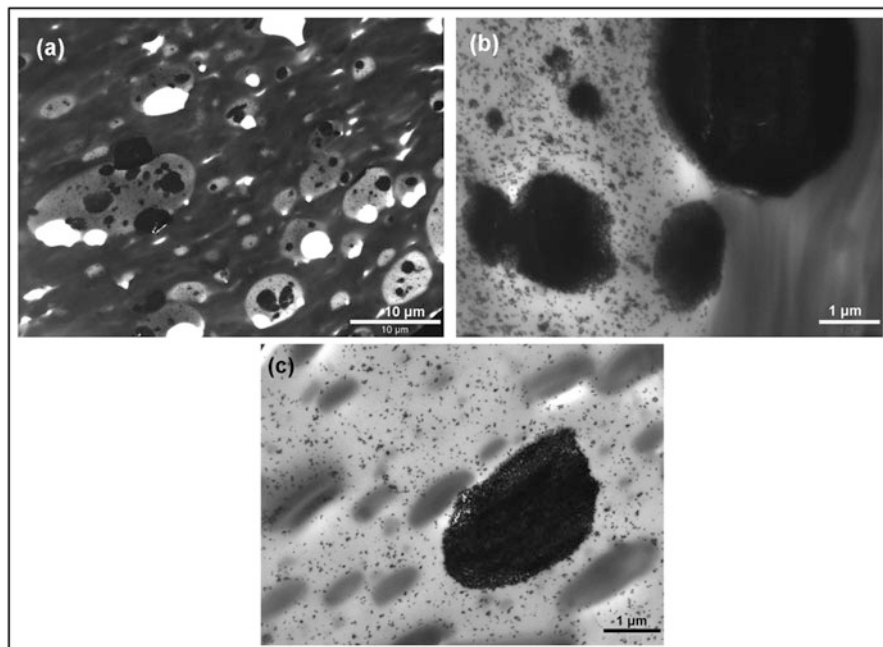


Fig. 31 TEM images of 30/70/5 w/w PLA/PCL/TiO₂ at (a) 1950 \times and (b) 13,500 \times magnification and (c) 70/30/5 w/w PLA/PCL/TiO₂ at 13500 \times magnification. (Reproduced with permission (Mofokeng and Luyt 2015))

materials have been described (Laredo et al. 2010; Jain et al. 2010; Eng et al. 2013; Xu et al. 2011). The prepared blends and nanocomposite morphological studies were carried out using TEM. TEM images of PLA/PCL blends with 5 wt% TiO₂ nanoparticles are shown in Fig. 31 in two distinct compositions. PCL was visible in the figure as the black phase that could be distinguished from the TiO₂ polymer phases. The majority of the nanoparticles was distributed in the PLA phase, and some small and large aggregates could be seen clearly in the pictures. However, there isn't a noticeable concentration of nanoparticles at the PCL and PLA interfaces.

The degradation products of neat PLA and 97/3 w/w PLA/TiO₂ are represented by their FTIR spectra in Fig. 32. It is evident that CO₂ intensity is higher between 2300 and 2400 cm⁻¹, at 1792 cm⁻¹, and at 325 °C for the C-O-C bands in the spectrum. The same bands have considerably lower intensities, at about 350 °C. At the same time, the other peaks such as at 2998 cm⁻¹, 2819 cm⁻¹, 2732 cm⁻¹, 2170 cm⁻¹, 2108 cm⁻¹, and 1371 cm⁻¹ completely disappeared. When TiO₂ nanoparticles were included, the carbonyl band shifted from 1792 to 1764 cm⁻¹ at around the same temperatures and lost intensity compared to pure PLA; the intensities were higher at higher temperatures. Strong evidence that the PLA interacted with nanoparticles and prevented their diffusion from the liquid polymer was obtained from these observations.

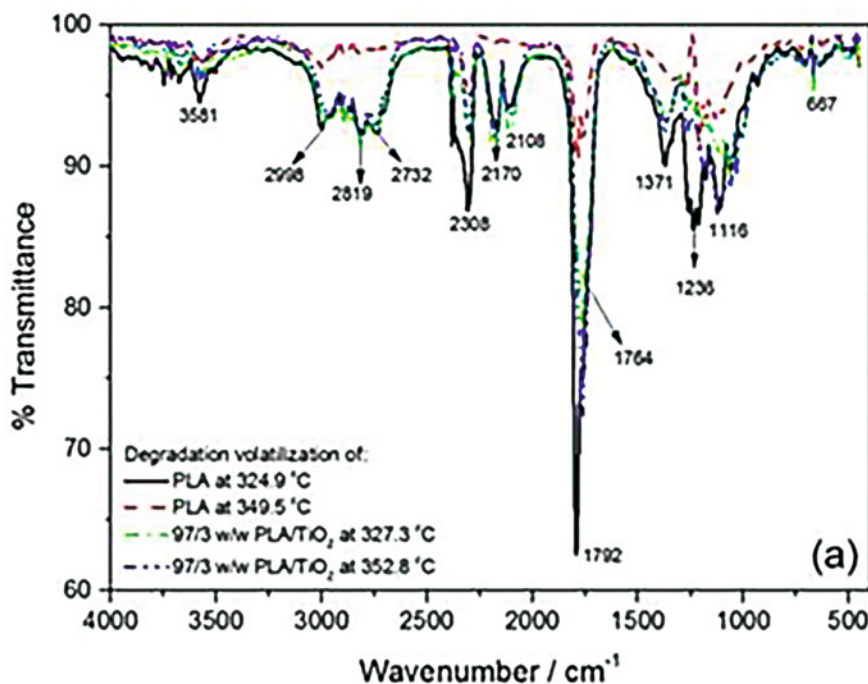


Fig. 32 FTIR spectra of (a) neat PLA and (b) 97/3 w/w PLA/TiO₂ degradation products. (Reproduced with permission (Mofokeng and Luyt 2015))

The degradation products of neat PCL and 97/3 w/w PCL/TiO₂ are depicted in FTIR spectra (Fig. 33). The hydroxyl (-OH) band is shown at around 3577 cm⁻¹. The carbonyl peaks obtained at (C=O) 1756 cm⁻¹ and the peak obtained between 2400 and 2300 cm⁻¹ correspond to carbon dioxide (CO₂). In contrast to the high intensities of pure PCL at higher temperatures, the overlap of the C-O-C, C-O, CH, and CH₂ bands between 1400 and 1100 cm⁻¹ is less than that of TiO₂ containing PCL at high temperatures.

The degradation volatiles of the mix and nanocomposites are shown in Fig. 34 as their spectra at 344 °C (the PLA degradation stage) and 396 °C, respectively (degradation stage of PCL). The previously described degradation products of PLA are represented by the peaks in the image that are characterized by lactide molecules, acetaldehydes with carbon monoxide, and oligomeric rings. The wavenumbers of the carbonyl peaks displayed in the degradation products from PCL and PLA correlate to the wavenumbers of the C-O peak's two peaks (1794 and 1758 cm⁻¹), which separate into two peaks. Two different wavenumbers were used to observe the CO₂ band (2375 and 2309 cm⁻¹). The spectra show different carbonyl and CO₂ bands corresponding to the breakdown products from each of the two polymers because PCL's degradation has been initiated and PLA's degradation is still underway.

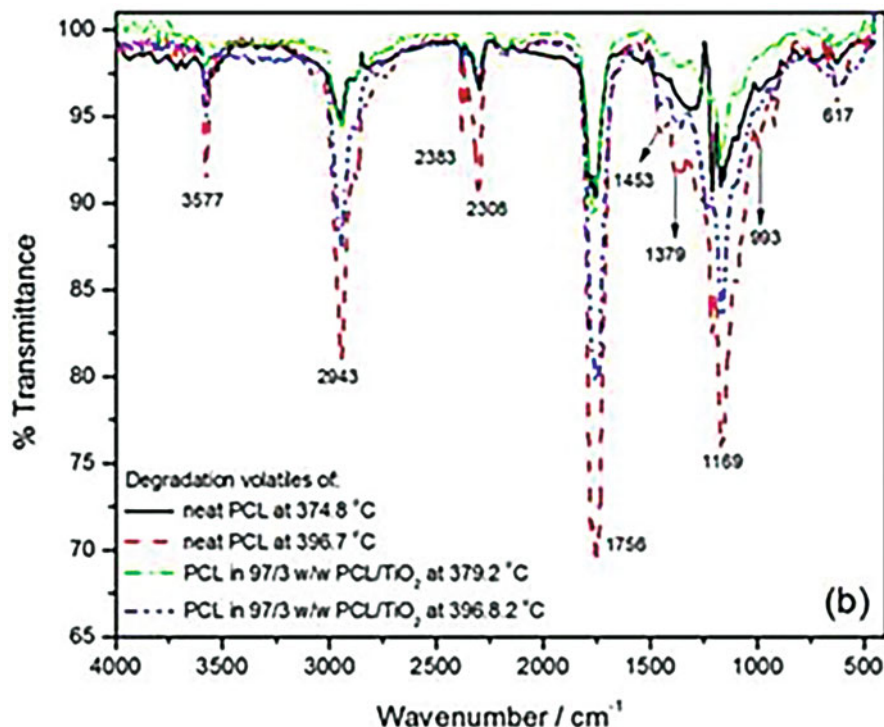


Fig. 33 FTIR spectra of (b) clean PCL and (97/3) w/w PCL/TiO₂ degradation products. (Reproduced with permission (Mofokeng and Luyt 2015))

Polycaprolactone shows excellent biocompatibility, biodegradability, and tensile strength. Due to its excellent resistance to slip, dislocation, and fatigue, it also shows significant potential in biomedical applications. Being able to overcome the limitations of ceramics, it can fulfil the critical need for medical materials (Abrisham et al. 2020). Kotcharat and his colleagues reported on the fabrication of aloe vera-loaded bacterial cellulose (BC) and polycaprolactone-based composites for wound dressing applications (Kotcharat et al. 2022). The composite material appeared as flat sheet and whitish in color. The structural confirmation was carried out using FTIR. Asymmetric and symmetric C-H stretching peaks are shown by the primary characteristic peaks of PCL at 2864 cm⁻¹ and 2949 cm⁻¹, respectively. Peaks at 1726 cm and 1296 cm⁻¹ are attributed, respectively, to carbonyl stretching and C-O and C-C stretching in the crystalline phase's backbone. The findings show that PCL was a significant portion of the composite's matrix phase. Increasing the aloe vera content revealed a striking increase in transmittance. From the morphological studies, the microstructure displays microcracks and porosity as the aloe vera was incorporated into the composite. The presence of voids was also observed, which is due to evaporation of solvent during the composite preparation. The characteristic peaks at 2θ values of 21°, 22°, and 24° matched with diffraction planes of (110), (111), and

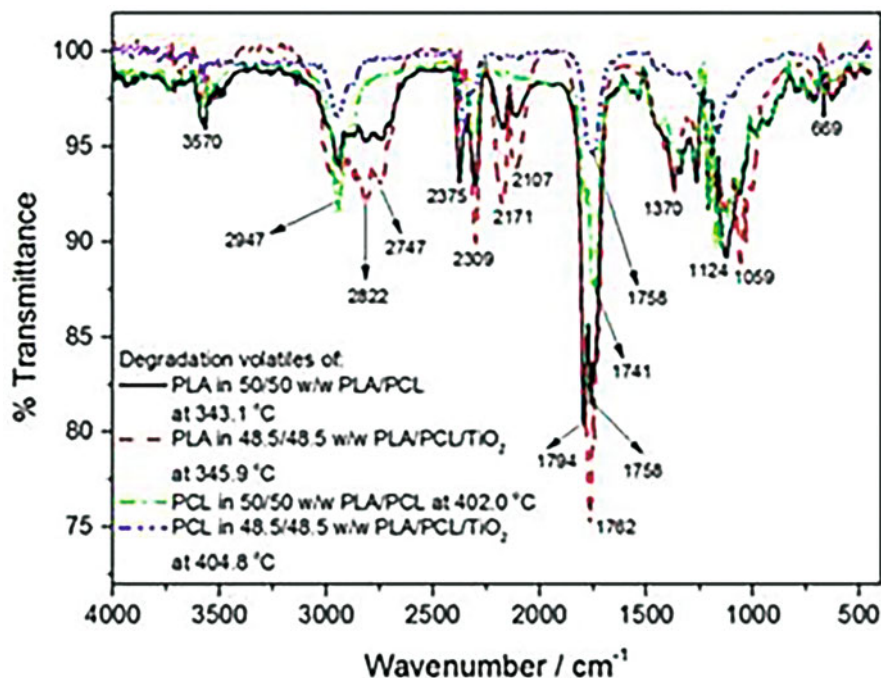


Fig. 34 FTIR spectra of the degradation products PLA and PCL in 50/50 w/w PLA/PCL and its nanocomposites with 3 wt%. (Reproduced with permission (Mofokeng and Luyt 2015))

(200), respectively, according to X-ray diffraction studies. No noticeable peaks were seen when the aloe vera was loaded into BC and PCL, most likely as a result of the reduced aloe vera concentration. Aloe vera, on the other hand, was incorporated into the composite's subsequent cellular foam, which reduced the crystallinity of the material. Aloe vera's amorphous phase hindered PCL's crystalline phase and had an impact on the composite film's tensile strength.

Conclusion

Due to its environmental benefits and the search for alternatives to petroleum-based resources, biopolymer-based research has significantly increased during the last few years. They are superior to other polymers due to their nontoxic nature, biodegradability, and biocompatibility and have a wide range of uses. In this chapter, we are highlighting the structural, morphological, and textural properties of some important biopolymers. A greater knowledge on biopolymers has resulted from the extensive study on structural and morphological investigation utilizing various microscopic techniques. Here, we are discussing some important biopolymer-based hydrogels, nanocomposites, and membranes using different methods. All the mentioned works

were focused on biomedical applications. The incorporation of biopolymers results in enhanced properties. Therefore, understanding the structural and surface properties of the biopolymers through these microscopic techniques and others will take great promises in all fields.

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Part IV

Biopolymers: Modifications



Tuning the Hydrophilic/Hydrophobic Behavior of Biopolymers

14

Surface Modifications

Reeba Mary Cherian, Hanieh Kargarzadeh, Noor Afizah Rosli, Cintil Jose, and Sabu Thomas

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R. M. Cherian

Department of Chemistry, Newman College, Thodupuzha, Kerala, India

School of Chemical Sciences, Mahatma Gandhi University, Kottayam, Kerala, India

H. Kargarzadeh (✉)

Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Lodz, Poland

e-mail: hanieh.k@cbmm.lodz.pl

N. A. Rosli

Department of Chemical Science, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia

C. Jose

Department of Chemistry, Newman College, Thodupuzha, Kerala, India

S. Thomas

School of Chemical Sciences, Mahatma Gandhi University, Kottayam, Kerala, India

School of Energy Materials, Mahatma Gandhi University, Kottayam, Kerala, India

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Abstract

Biopolymers gained increasing attention in various fields due to their versatile properties such as renewability, biodegradability, sustainability, eco-friendly, and because they possess good mechanical properties. However, some of the intrinsic properties of biopolymer such as wettability limit their industrial application. Modification (physical/chemical) of biopolymers is an accepted technique to tune their surface properties and consequently increase the potential application. This chapter summarizes some of the recent advances in various surface modification techniques, modifying agents, and functionalization of biopolymers (natural and synthetic) as well as their physicochemical properties before and after modification.

Keywords

Biopolymers · Surface modification · Functionalization · Physio-chemical properties

Introduction

The growing environmental awareness has become an important factor in recent decades and resulted in increased attention to the development and application of biopolymers. The term “biopolymer” applies to various thermoplastic and thermoset polymers derived from renewable resources or fossil fuels. It is also applied to biodegradable polymers. Biopolymers are classified into two major groups: natural biopolymers (polysaccharides, proteins, polypeptides, etc.) and synthetic biopolymers (polylactic acid, poly hydroxybutyrate, polybutylene succinate, etc.). Biopolymers can either be hydrophilic or hydrophobic by their chemical structure but most of them are hydrophilic due to the presence of surface hydroxyl groups especially plant fibers. But this intrinsic character sometimes could turn into a challenging factor especially in the application stage, for example, in the preparation of composites with hydrophobic matrix, there occurs a decrease in the interfacial adhesion. Hence the need of tuning the surface properties is an essential criteria as per the need.

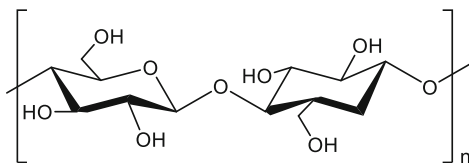
The different types of surface modification of the biopolymers with appropriate modifying agents can induce hydrophobic or hydrophilic properties. The reactive functional groups in the chemical structure of these biopolymers serve this purpose. The modifying agents can simply be a biopolymer with the desired property or biological enzymes or the agents should contain a reactive site with either a long chain hydrocarbon or a simple hydrocarbon to induce hydrophobicity and hydrophilicity or simple oxidation as per the application. The selection of an appropriate modifying agent is challenging because even though the tuning of wettability property is the main factor, we should also consider other factors like thermal degradability, mechanical properties, cost-effectiveness, cytotoxicity, etc. in different disciplines of application. Hence, this could turn out to be a challenging brainstorming matter. The selection of the modifying technique is also equally challenging. There are different chemical, physical, and environmentally friendly green modifying agents and techniques reported.

Researchers are in constant search to optimize the best agents to serve different purposes with cost-effective procedures because the effectiveness of these agents in one dimension could be a disadvantage in the other dimension. This chapter briefly explains the sources, production or synthetic technique, and physio-chemical properties of natural and synthetic biopolymers and discusses the different types of modifying agents and the techniques used.

Natural Polymers

The term “natural polymers” is an accepted definition for the polymers found in plants, animals, and microorganisms, and composed of either inorganic or organic subunits. The following section describes the most abundant and common natural polymers from three major groups of polysaccharides, proteins, and polyphenols.

Scheme 1 Chemical structure of cellulose



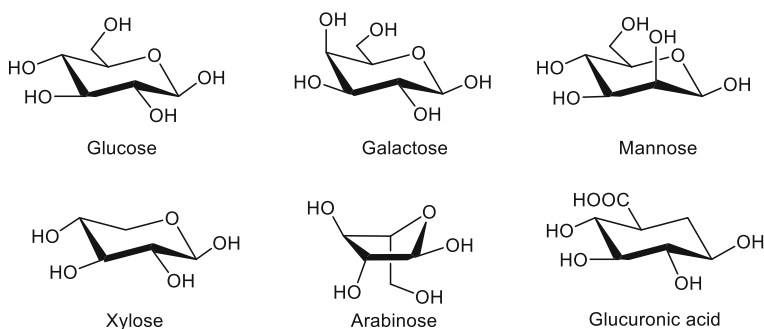
Cellulose

Cellulose is the most abundant polysaccharide on Earth. It exists in plants, animals, and some bacteria. It is a linear semicrystalline polymer, made of the repeat unit of anhydro-D-glucopyranose connected by $\beta(1 \rightarrow 4)$ -glycosidic bonds (Scheme 1). Cellulose has a very organized structure in wood fiber due to its linear structure and intermolecular hydrogen bonding. Cellulose fibrils consist of alternating less ordered amorphous and high ordered crystalline phases. The amorphous part is more reactive than the crystalline part due to the low amount of intermolecular interactions which ease the accessibility of the reagents. The degree of polymerization of cellulose is in the range of 10,000 to 15,000 depending on the source. Cellulose crystallite exists in four major polymorphic forms, I, II, III, and IV. Native cellulose I exists in the crystalline form of I_α containing a triclinic one chain unit cell and I_β containing a monoclinic two chain unit cell. The other three crystalline allomorphs are attributed to modified cellulose. For example, cellulose II can be obtained via alkali treatment.

Due to its high crystalline structure, cellulose is insoluble in water and some high polarized solvents. However, the presence of hydroxyl groups on its polymer chain makes it highly hydrophilic. Various chemical and physical modification techniques have been used to modify the hydrophilicity of cellulose and its functionalization. The hierarchical architecture of natural cellulose consisting of nanoscale fibrils and crystallites allows the extraction of the nanocomponents via mechanical and chemical methods or through a combination of both of these techniques. Cellulose nanocrystal and cellulose nanofibers with different geometry and properties can be extracted from different sources of cellulose. They have gained extensive attention in the past decades due to their renewability, biodegradability, nontoxicity, low weight, and high mechanical properties. These two new classes of nanomaterials are used in a wide range of high-performance products such as food, medicine, clothes, cosmetics, papers, composites, and packaging (Kargarzadeh et al. 2017, 2018).

Hemicellulose

Hemicellulose is a heteropolysaccharide with a branched structure present in the primary and secondary cell walls of wood and annual plants. Hemicellulose has an amorphous structure with low molecular weight which makes it soluble or partially soluble in water. Unlike cellulose in which the monomer units are chemically



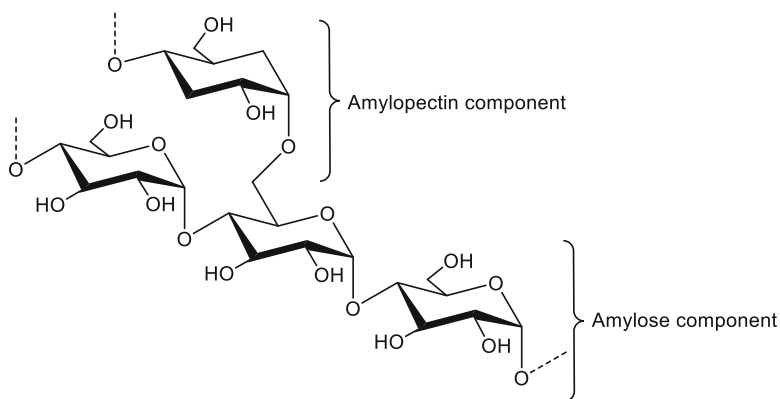
Scheme 2 The main monosaccharides of hemicellulose

homogeneous, hemicellulose consisted of mixed monomers of sugars such as glucose, xylose, mannose, galactose, arabinose, fucose-glucuronic acid, and galacturonic acid. The hemicellulose composition, chemical structure, and abundance vary widely between different species and cell types. For example, the prominent hemicellulose in softwood, plant seed, and fruits are arabinose, galactose, and mannose, whereas the main hemicellulose monosaccharide in hardwood and grasses is xylose (Scheller and Ulvskov 2010; Zhang et al. 2021). The chemical structure of main hemicellulose monosaccharides is shown in Scheme 2.

Hemicellulose can be extracted from lignocellulosic biomass via several techniques such as hydrothermal extraction, alkaline treatment, dilute acid hydrolysis, auto-hydrolysis, and steam explosion. Among them dilute acid hydrolysis is the most common technique that produces a high quantity of monomeric sugars. Auto-hydrolysis and steam explosion produce oligomer form of hemicellulose while alkaline treatment extracts hemicellulose in polymeric form. Hemicellulose is highly hydrophilic due to a large number of hydroxyl groups on its backbone and side chains. Esterification, etherification, graft copolymerization, and other reactions can be conducted to chemically modify these hydroxyl groups. The chemical modifications convert the free hydroxyl group to other functional groups which tune various properties such as hydrophobicity, thermal stability, and solubility in different solvents. The modified hemicellulose can find applications in various fields such as composites, food packaging, coating, filtration and adsorbent.

Starch

Starch is a semi-crystalline plant-driven polysaccharide. It serves as an energy reserve in a variety of plants such as fruits, grain seeds, tubers, and roots. It is a copolymer with a complex structure consisting of 20–30% of amylose, which is a linear polysaccharide of α -D-glucose units linked α -(1 \rightarrow 4) with a molecular weight of 10^3 – 10^6 Da, and amylopectin which is a high molecular weight 10^7 – 10^9 Da branched polymer. Amylopectin is the main composition of starch and consists of α -D-(1 \rightarrow 4)-glucose units and α -D-(1 \rightarrow 6)-glucose units in the branched point

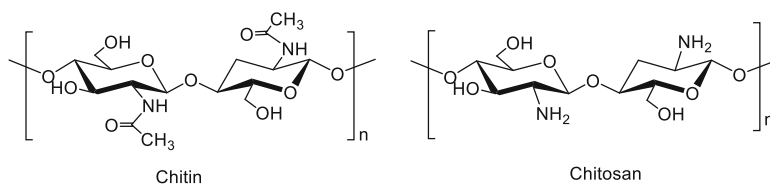


Scheme 3 Chemical structure of starch with amylose and amylopectin components

(Scheme 3). The starch granule size, shape, structure, and ratio of amylose and amylopectin, and the degree of crystallinity depend on the origin of starch (Smith 2001). Amylopectin is the main crystalline component of starch with a degree of polymerization around 10–20 which is insoluble in water and produces soft gel and weak film when disperse in water, whereas amylose is the amorphous component that produces tough gel and strong film with the tendency to retrograde. The structure of starch granules is well described by Bertoft (2017). Due to its gelling and film-forming properties as well as its biodegradability and biocompatibility, it has been widely used in various applications such as food, packaging, cosmetics, and drug delivery (Rodrigues and Emeje 2012; Yazid et al. 2018; Qamruzzaman et al. 2021). However, native starch is unable to attain specific industrial requirements which restricted its application. The chemical or physical modification is therefore proposed to improve the properties of starch. Crosslinking, acetylation, esterification, oxidation, and acid hydrolysis, ultrasonication, heat moisture treatment, dry heat treatment, pulsed electric field, and plasma are some examples of starch modification which is comprehensively discussed in the following publication (Chen et al. 2015; Zhu 2017; Zia-ud-Din and Fei 2017; Yazid et al. 2018; Amaraweera et al. 2021; Maniglia et al. 2021).

Chitin and Chitosan

Chitin is the second most abundant polysaccharide after cellulose. It is nontoxic, nonallergic, antimicrobial, and biodegradable. It is the primary component of the exoskeleton of arthropods such as crustaceans, cell walls in fungi, and insects. It is an amide derivative of glucose with a linear structure composed of β-(1–4)-linked *N*-acetyl-D-glucosamine units (Scheme 4). Chitin is hydrophobic and insoluble in water and organic solvents (Rinaudo 2006). Its average molecular weight is $1\text{--}2.5 \times 10^6$ Da with a degree of polymerization of approximately 500–10,000 and a degree of acetylation of more than 90% (Mourya and Inamdar 2008). The crystallinity and thermal



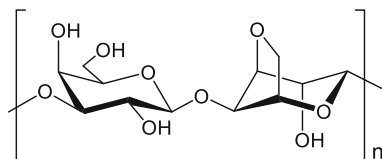
Scheme 4 Chemical structure of chitin and chitosan

properties of chitin are determining factors for its various potential applications (Villette et al. 2002; Rihaudo 2006). The high crystallinity and mechanical properties of chitin and its nanoparticles make them also suitable candidates for reinforcement in polymer composites. However, its insolubility limits its application. If the degree of deacetylation (DA) of chitin reaches about 50%, it becomes soluble in aqueous media and is called chitosan (Rihaudo 2006). Chitosan can be prepared in different forms such as film, powder, gel, paste, fiber, and nanoparticles (Ahmed and Ikram 2016). Its solubilization occurs by protonation of the basic amino group on the C-2 position of the D-glucosamine. Chitosan is a linear semicrystalline polysaccharide and can be prepared with different molecular weights and DA from chitin. The average MW of chitosan is about 100–500 kDa which is lower than the MW of chitin. The chitosan chemical structure is composed of β -(1–4)-linked glucosamine and *N*-acetyl glucosamine units. Similar to chitin, it is almost insoluble in water (except in acidic pH) and highly crystalline which limits its application to some extent. Therefore, many techniques are applied to improve the solubility of chitosan such as chemical and enzyme modification (Kaczmarek et al. 2019; Wang et al. 2020). Both hydroxyl and amine groups of chitosan are reactive groups that ease the modification and functionalization of chitosan. Effective chemical modifications expand the application of chitosan compared to its natural compound. For example, acetylation and etherification of chitosan enhance its water solubility, biocompatibility, anticoagulability, and blood compatibility which can be used as a carrier or sustained release agent in the pharmaceutical application (Furlani et al. 2019). Alkylation also improves the solubility of chitosan; however, due to its hydrophobicity, the solubility is low depending on the length of the alkyl chain (Burr et al. 2018). More examples of the effect of various chemical modifications on the properties of chitosan and its applications can be found in (Negm et al. 2020; Wang et al. 2020; Saheed et al. 2021). Due to the biodegradability, antimicrobial, non-toxicity, antioxidant activity, and functionality, both chitin and chitosan are investigated in many areas of application such as tissue engineering, drug delivery, stabilizing cosmetic ingredients, packaging, and membrane for removal of metal ions, dyes, and pigments in wastewater (Rinaudo 2006; Khoushab and Yamabhai 2010; Ahmed and Ikram 2016; Wang et al. 2020).

Agar

Agar is a natural polysaccharide with strong gelling properties. It is occurring in the cell wall of various red seaweeds where they promote flexibility and mobility to the plant for easy adaption to different marine environments (Matsushashi 1990). Agar

Scheme 5 Chemical structure of agarabios, repeating unit of agar

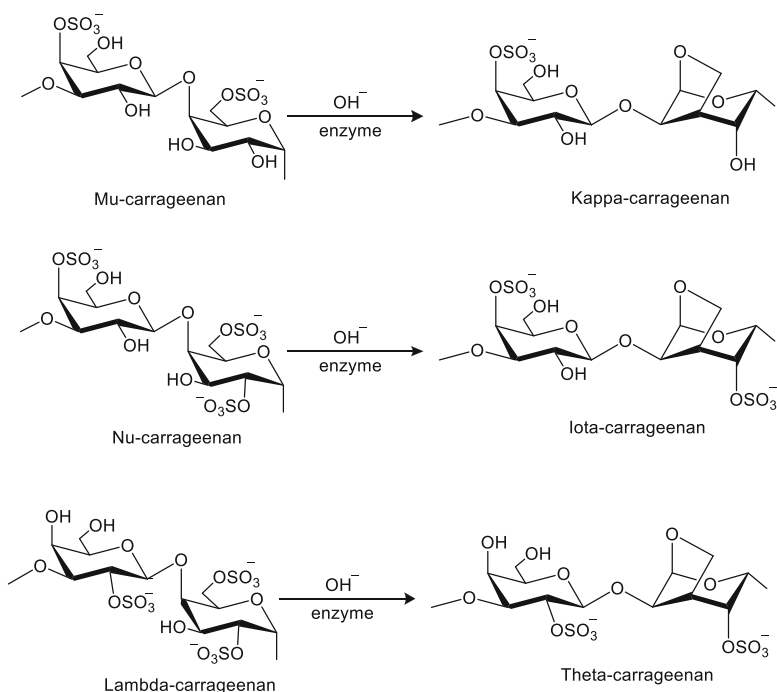


consists of two different polysaccharides, agarose, and agaropectin. Agarose which is the gelling fraction of agar is a linear polysaccharide consisting of alternate D-galactose and 3, 6 anhydro-L-galactose (Scheme 5). It has a high molecular weight ($>100,000$ Da) and low sulfate content ($<0.15\%$), whereas agaropectin has a lower molecular weight ($<20,000$ Da) and higher sulfates content (5–8%). The agarose backbone may contain sulfate, urinate, pyruvates, or methoxy groups (Lee et al. 2017). The typical ratio of agarose and agaropectin is 70:30 (Zeece 2020). However, the ratio of agarose and agaropectin is varied from one species to another and depends on the species, the season, the environment it grows in, the extraction method, etc. The higher ratio of agarose to agaropectin results in higher gel strength (Padmesh and Singh 2021).

Agar is water-soluble. A small amount of 1% agar in water is good enough to form a rigid gel suitable for most applications. Agar is a physical gel and translucent, which does not require any other substances to gel. Unlike the chemical gels with the covalent bond, the agar gelation process is thermally reversible and occurs exclusively by hydrogen bonds. Due to this unique gelling property, it holds a great amount of water in the interior network which can freely move through the macroreticulum (Matsushashi 1990; Armisen et al. 2009). The gelling properties and gelling condition of agar depend on the functional groups on its backbone, for example, the gel setting temperature of agar depends on the level of methoxy content, and the low methoxy contents give the lower gel setting temperature and vice versa. Alkali pretreatment increases the anhydrous bridges within the agar molecules, consequently increasing gel strength, gelling, melting temperature, and viscosity (Yarnpakdee et al. 2015). Agar has wide application mainly in food industry for production of puddings, custards, soft candies, etc. It is also added to baked goods to inhibit staling and improves the texture of processed cheese and frozen desserts. Agar has similar properties to gelatin which makes it a potential substitute for animal-based gelatin in vegetarian food (Lestari et al. 2019). Packaging (Wang and Rhim 2015) and tissue engineering (Salati et al. 2020) are other applications of agar.

Carrageenan

Carrageenan is a linear sulfated polysaccharide and originated from red seaweed. The chemical structure of carrageenan consists of 3-linked β -D-galactopyranose and 4-linked α -D-galactopyranose or 4-linked 3,6-anhydro- α -D-galactopyranose unites (Scheme 6). Based on the chemical structure and position of sulfate ester and the presence of anhydro group, carrageenan is classified into six types, kappa, iota,



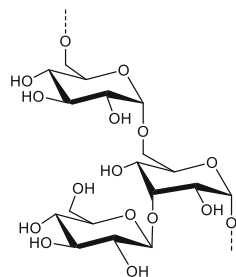
Scheme 6 Chemical structure of the repeating units of carrageenan

lambda, mu, nu, and theta. The molecular weight of carrageenan is in the range of 100 to 1000 kDa. It is soluble in hot water, partially soluble in cold water, and insoluble in organic solvents, oil, and fats. The water solubility of carrageenan depends on the level of hydrophilic sulfate groups and their associated cations. Among all types of carrageenan, kappa and iota possess strong gel properties, whereas, lambda-carrageenan is characterized as a thickener agent. Similar to agar, the gelation behavior of carrageenan in aqueous solution is thermally reversible and depends on the temperature, concentration of carrageenan, and species of cations, typically potassium or calcium which are essential agents for gelation to proceed. Gelling mechanism of carrageenan is described in Campo et al. (Campo et al. 2009). Potential applications of carrageenan in biomedical (Deng et al. 2018), electrode devices (Sangeetha et al. 2020), composites, and food industry (Hotchkiss et al. 2016) were reported.

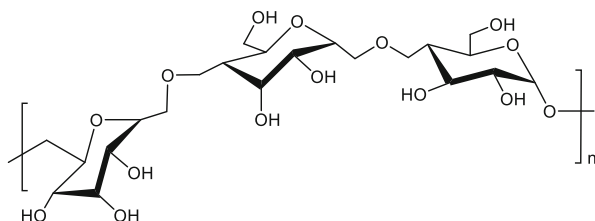
Dextran

Dextran is a bacterial polysaccharide produced by lactic acid bacteria, mainly by *Leuconostoc mesenterioide*. It is branched and its main chain consists of α -(1 \rightarrow 6) linked D-glucose units in which α -(1 \rightarrow 6) linkage varies from 50 to 97% of the total glycosidic bonds. The branches mainly take place in the position α -(1 \rightarrow 2), α -(1 \rightarrow 3),

Scheme 7 Chemical structure of dextran repeating units



Scheme 8 Chemical structure of pullulan



and/or α -(1 \rightarrow 4) (Scheme 7). The differences in the size and chemical structure of dextran as well as its molecular weight (from 3000 to 2,000,000 Da) depend on the dextranase produced by the bacterial strain (Varshosaz 2012; Wang et al. 2017). Dextran is hydrophilic and water-soluble; however, the branch linkages pattern of dextran determines its solubility. The presence of >43% branching via 1,3- α linkage leads to enhance the water insolubility of dextran, while the presence of 95% linear linkage makes dextran water-soluble (Purama et al. 2009). The presence of reactive hydroxyl groups on the chemical structure of dextran eases its chemical and physical functionalization. The properties of dextran are changed, depending on the degree of substitution of functional groups. For example the high degree of substitution of hydrophobic functional groups makes the dextran water insoluble and nondegradable by the enzyme (Aumelas et al. 2007; Wang et al. 2016). The water-solubility and its functionability, biodegradability, biocompatibility, and nontoxicity make dextran in both nano and micro size a suitable material in drug delivery application (Huang and Huang 2018; Elalmis et al. 2022), food industry (Kothari et al. 2014; Lazić et al. 2020; Wang et al. 2021), and cosmetics (Baptista and Freitas 2021).

Pullulan

Pullulan is a linear microbial polysaccharide produced by a yeast-like fungus, *Aureobasidium pullulans*, in a fermentation medium. It consists of α -(1 \rightarrow 6)-repeated maltotriose units linked α -(1 \rightarrow 4) glycosidic bond (Scheme 8). Its chemical structure and molecular weight depend on pH, carbon source, producing

microorganism, and fermentation condition (Singh et al. 2015). The polydispersity of pullulan is between 2.1 and 4.1 (Wiley et al. 1993) which is significantly lower than the polydispersity of dextran and amylose which is due to the different cell morphology-regulated mechanism or the biosynthesis pathway of pullulan formation (Shingel 2004). Pullulan is soluble in water and dilute alkali and insoluble in alcohol and other organic solvents except dimethylsulfoxide and formamide (Prajapati et al. 2013). However, its solubility limits the application of pullulan. The solubility of pullulan can be decreased by chemical modification, polymer grafting, and blending with other polymers (Shingel 2004; Babu et al. 2013). Pullulan is a nonionic polysaccharide, colorless, tasteless, non-toxic, edible, non-mutagenic, non-carcinogenic with good film and fiber formability. These unique properties make it a suitable material for food coating and packaging, cosmetic, biomedicine and drug delivery, and water purification (Ghosh et al. 2022). Due to the unique structure of pullulan, its nanoparticle consists of both hydrophobic and hydrophilic characteristics which increase its utility in the field of pharmaceuticals (Singh et al. 2015).

Collagen and Gelatin

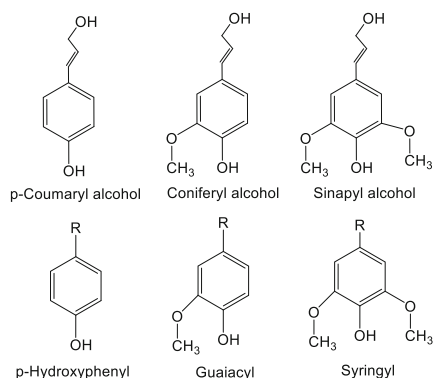
Collagen is the most abundant protein found in the tissues of animals. The main natural sources of collagen are pig skin, pork and cattle bones, and bovine hide while it can be also obtained from nonmammalian species (Gómez-Guillén et al. 2011). Collagen is made up of amino acids, such as glycine, proline, arginine, hydroxyproline, lysine, alanine, aspartic, glutamic, and hydroxylysine, of which glycine is the main amino acid. Collagen is classified into two main groups, fibrillary and non-fibrillar consisting of 29 different types based on the differences in structure, location, and properties (Owczarzy et al. 2020). Collagen with a molecular weight of 300,000 is insoluble in water. Its solubility can be improved by enzyme, acid, or alkali treatment. Native collagen possesses high mechanical properties, which are lost when it became water soluble (Figueiró et al. 2004).

Gelatin is hydrolyzed collagen with a lower molecular weight than collagen, and the amino acid structure. It is soluble in hot water, but if the gelatin solution boils for a long time, it changes its properties due to decomposition and would not be reformed after cooling. While at a temperature below 35 °C to 40 °C it is gelatinous (Williams and Phillips 2003; Raja Nhari et al. 2011). Gelatin has excellent physical properties such as high dispersibility and dispersion stability, low viscosity, and high water retention as well as high toughness. It is an important material in food industries where it is used as a thickener and foaming agent, emulsifiers, clarifiers, and dispersants. Both collagen and gelatin can be modified chemically and physically, blend with other polymers, or cross-linked to obtain desired properties (Erikson et al. 2008; Zhang et al. 2010; Sionkowska 2021). Both collagen and gelatin have a large range of applications in biomedical, drug delivery, and food and beverage industries (Hashim et al. 2015; Ghomi et al. 2021; He et al. 2021; Luo et al. 2022).

Lignin

Among natural polyphenols, lignin is the most abundant polymer on Earth. It is the third major wood cell wall component and is covalently bonded to polysaccharides. Lignin serves as a block of cement between wood fibers, as a stiffening agent within the fibers, and as a barrier to the enzymatic degradation of the cell wall (Goldstein 2004). It is an amorphous complex polymer with a three-dimensional cross-linked network of phenylpropane units with high molecular weight. Lignin is relatively hydrophobic and its chemical structure consists of three monolignols – p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, and also consists of three types of phenylpropanoid – p-hydroxyphenyl, guaiacyl, and syringyl (Scheme 9). These reactive components are attached by a series of characteristic linkages, such as α -O-4, β -O-4, β -5, and β - β' . Similar to polysaccharides, the chemical structure of lignin and the ratio of monolignol and intermolecular linkages depend on its source (Duval and Lawoko 2014; Spiridon 2020). For instance, hardwood lignin mainly consists of coniferyl alcohol and sinapyl alcohol, while the major monolignols component in softwood lignin is coniferyl alcohol and grass lignin has three types of monolignols (Gellerstedt and Henriksson 2008). In addition, softwood and hardwood lignins differ in methoxyl content and in the degree of crosslinking. Hardwood lignins have more methoxyls, which reduce the degree of crosslinking and block potential reactive sites (Goldstein 2004). The molecular weight of lignin depends on the extraction method. Klason lignin has MW from 260 to 50 million and Bjorkman lignin has MW of 11,000 (Rowell et al. 2012). Lignin is obtained as a by-product of the paper and lignocellulosic industries. Based on the production process, lignin is classified into Kraft lignin, Soda lignin, Lignosulphonates, Organosolv lignin, steam explosion lignin, and enzymatic hydrolysis lignin (Vishtal and Kraslawski 2011). Each of the lignin classes possesses different chemical functionality and properties. However, all classes of lignin have many unique properties such as UV absorbance, antioxidation, and antimicrobial activity which is attributed to the presence of phenolic, ketone, conjugated double bonds, and other

Scheme 9 Chemical structure of monolignols and their corresponding phenylpropanoid



chromophore groups (Espinoza-Acosta et al. 2016; Lin et al. 2021; Zhang and Naebe 2021). Lignin has potential applications in polymer industries. However, its application is limited due to its thermal degradation and mechanical properties. Different modification techniques have been proposed to improve the chemical reactivity and properties of lignin. For instance, oxidative pre-treatment of lignin increases its thermal stability (Brodin et al. 2010). The sulfonation treatment increases the water solubility of kraft lignin (Konduri and Fatehi 2015). Alkylation treatment improves the compatibility of lignin with hydrophobic polymers like polypropylene (Barkoula et al. 2008). Other lignin modification techniques such as methylation, esterification, carboxymethylation, and polymer grafting have been also reported in the literature (Jardim et al. 2020). Lignin in both micro and nanoscale can be used as reinforcement in polymer composites (Kargarzadeh et al. 2020) or as surfactant, emulsifier, and plasticizer (Österberg et al. 2020). It has a good capacity to adsorb heavy metal ions and thus possesses potential application for water purification application (Thakur et al. 2017). Lignin also shows potential applications in food, cosmetic, and pharmaceutical industries as an antioxidant agent instead of synthetic additives (Widsten et al. 2020; Chaubey et al. 2021).

Synthetic Biopolymers

The term “synthetic polymer” refers to chemically created polymers that are either manufactured from renewable sources or selectively subjected to different types of degradation, such as heat, photo, and corrosion degradation. Therefore, the degradation characteristics of the polymers are crucial in understanding the term “synthetic biopolymer,” notwithstanding variations in the sources of the monomers used. For instance, poly- ϵ -caprolactone (PCL), a biopolymer derived from petroleum, can undergo total degradation. On the other side, there is a non-degradable biopolymer made from bio-based materials, like bio-polyethylene (bio-PE). Therefore, these variables must be considered when discussing biopolymers. Figure 1 provides a more precise illustration of the categorization of synthetic biopolymers. Polylactic acid (PLA), polyhydroxybutyrate (PHB), polyglycolic acid (PGA), polycaprolactone (PCL), poly(butylene succinate) (PBS), poly(butylene-adipate-co-terephthalate) (PBAT), and poly(hydroxyalkanoates) (PHAs) are the main synthetic biopolymers. Each of these is covered in detail in the following sections.

Polylactic Acid

Over the past few decades, researchers have focused the most on PLA, a thermoplastic polyester (Ray and Okamoto 2003; Tang et al. 2012). It is made up of repeating lactic acid units, as in Scheme 10. This lactic acid is typically produced by converting sugar or starch from plant sources like corn, wheat, or rice via bacterial fermentation or petrochemical methods.

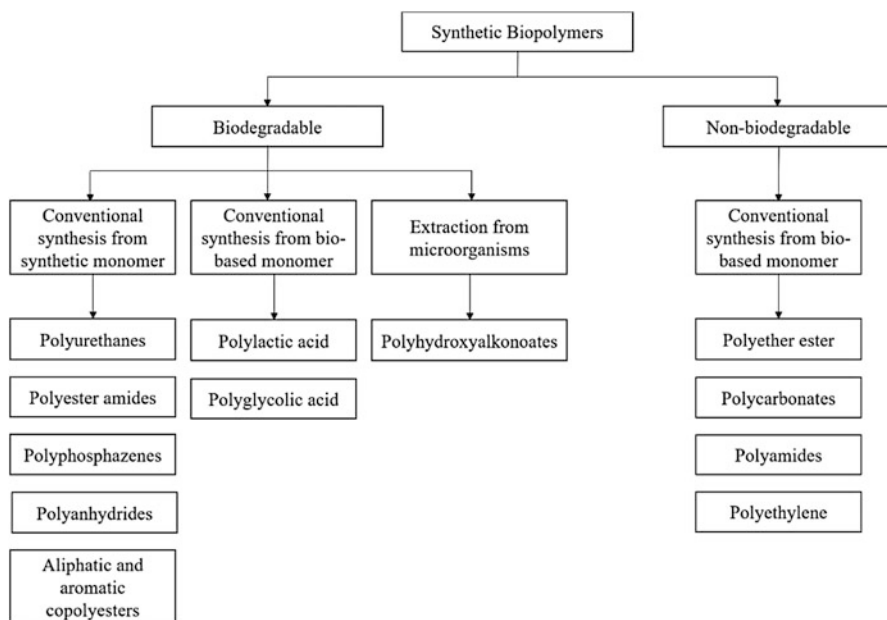
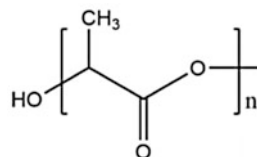


Fig. 1 Synthetic biopolymer classification based on biodegradability. (Reprint with permission from (George et al. 2020))

Scheme 10 Repeating unit of PLA



Compared to other biopolymers like PHA, PEG, PCL, etc., PLA has more excellent heat processing and energy savings (Vink et al. 2003). Its elastic modulus and tensile strength are equivalent to poly(ethylene terephthalate) (PET). It is a biopolymer that is environmentally friendly and has outstanding biocompatibility, but it also has several drawbacks that restrict where it may be applied. PLA is a highly fragile polymer, breaking with less than 10% elongation. PLA's hydrolytic degradation rate is generally slow and greatly influenced by the substance's crystallinity, molecular weight, morphology, and water permeability (Rosli et al. 2021). The hydrophobic characteristic of PLA, which exhibits a static water contact angle of around 80 °C, accounts for its slow degradation rate. Furthermore, because of its less reactive side-chain group, PLA is chemically inert, making bulk modification a difficult procedure.

Numerous techniques, including copolymerization and blending, are used to boost the usage of PLA in various applications to enhance its toughness capabilities

(Chang et al. 2015; Kong et al. 2018). PLA is blended with various thermoplastics and elastomers (Bitinis et al. 2011; Armentano et al. 2015), and as a result, its toughness increases in addition to its degradation rate (Rosli et al. 2018). The polymerization of PLA with various monomers was also performed to increase the toughness and degradation rate of PLA. For instance, due to the hydrophilic and biocompatible properties of PEG, the copolymerization of PLA with PEG is widely reported. Consequently, PLA's toughness and degradation rate successfully improved through PEG's copolymerization (Han et al. 2016).

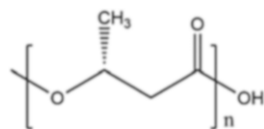
In addition to bulk modification, PLA surfaces may be modified; these modifications are classified as permanent (covalent bonding) and non-permanent. Entrapment, coatings, migratory additives, and plasma treatment are a few examples of non-permanent alterations to PLA surfaces. Meanwhile, photografting and wet chemistry are used in the chemical conjugation process to modify PLA's surface permanently. All of these methods effectively increase the hydrophilic properties of PLA (Mann et al. 2019; Varga et al. 2019; Wu et al. 2022).

Poly Hydroxybutyrate (PHB)

Poly (hydroxybutyrate) (PHB) is a biologically formed product and is the largest class of PHAs. PHBs are natural polymers in some bacteria and algae as intracellular storage materials. They are extracted from the corresponding cell biomass utilizing the proper downstream processing processes (Raza et al. 2020). If the alkyl (R) group in PHAs is methyl, it will produce the structure of PHB as shown in Scheme 11. PHB displayed above 50% ordered crystallinity and a melting point of 180 °C. Due to this, PHB displays high brittle properties much as PLA. PHB's hydrophobic characteristics also cause it to degrade slowly (Abid et al. 2016).

Based on the desired properties, bulk modification of PHB is done through blending techniques with synthetic or natural polymers. The mechanical and physicochemical characteristics of PHB are often successfully enhanced after blending (Parra et al. 2006). PHB's surface may be modified in two ways: by modifying the functional group physically or chemically and via coating (Bu et al. 2019). PHB can have its surface chemically modified by aminolysis, hydroxylation, methylation, or chlorination (Raza et al. 2018; Yeo et al. 2018). Chemical treatments on the surface of PHBs provide new active functional groups that can enhance the interaction of PHBs with other molecules (Wei et al. 2015; Kai et al. 2019).

Scheme 11 Repeating unit of PHB



Polyglycolic Acid/Polyglycolide (PGA)

Poly(glycolic acid) (PGA), a valuable biopolymer due to its high cost and lack of a technique for cost-effective big-scale manufacturing, is a member of the poly(α -hydroxy acids). Due to its high crystallinity (45–55%), PGA is insoluble in many solvents (Budak et al. 2020). Compared to PLA, it degrades more quickly and has stronger mechanical properties. In addition, when compared to other polyesters, PGA is the most hydrophilic. Glycolic acid and glycolide are employed as monomers to manufacture PGA (Scheme 12).

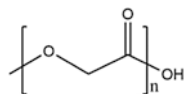
Although the hydrophilic properties of PGA are among the highest compared to other polyesters, however, synthetic aliphatic polyesters are often not chemically modifiable and are pretty hydrophobic. Chemical modification, such as grafting, which involves adding various new functional groups to the PGA backbone, is one of the modification techniques that can be used (Zhang et al. 2013). Another approach to modifying the hydrophobic properties of PGA is through the simple coating and covalent bonding of ligands on the surface of PGA (Fu and Zhang 2020).

Poly- ϵ -caprolactone (PCL)

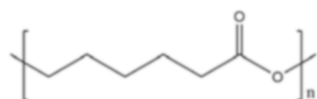
An aliphatic polyester made of hexanoate repeating units is called poly(ϵ -caprolactone) (PCL), as depicted in Scheme 13. Most PCL's properties rely on the degree of crystallinity and molecular weight. It is naturally hydrophobic and highly flexible, with an elongation at break of 1100% (Matzinos et al. 2002). PCL is a unique biopolymer since it frequently forms miscible and compatible blends when combined with other polymers (Labet and Thielemans 2009). However, PCL's poor solubility, mechanical strength, and stability limit its usage in various applications (Dash and Konkimalla 2012).

PCL can be modified physically (blend and composite) and chemically (copolymerization and grafting) to improve or add specific properties to PCL. Physical modifications are usually performed to improve the mechanical properties of PCL without altering the backbone of the polymeric chain (Olgun et al. 2011). Contrarily, chemical modification involves changing the PCL backbone by introducing different

Scheme 12 Repeating unit of PGA



Scheme 13 Repeating unit of PCL



functional groups. Typically, hydrophobic PCL is changed chemically to add hydrophilic properties; consequently, the modified PCL has amphiphilic properties (Hu et al. 2012).

Poly(Butylene Succinate) (PBS)

Poly (butylene succinate), an aliphatic polyester formed from succinate, is not considered bio-based because it is made from traditional fossil fuels. However, it can also be made utilizing monomers from renewable sources (Jansen and van Gulik 2014). PBS is often made by reacting a diacid or acid anhydride with diols, and Scheme 14 illustrates the recurring molecules of PBS.

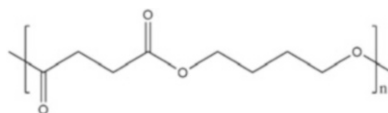
PBS demonstrates good biodegradability, mechanical properties, thermal stability, and heat deflection. PBS is typically combined with other polymers or fibers to address some drawbacks, including excessive softness, poor gas-barrier properties, and low viscosity (de Matos Costa et al. 2020). The hydrophobic characteristics of PBS can also be modified via photografting and plasma methods, which involve introducing hydrophilic substances into the molecular structure of PBS (Mizuno et al. 2015).

Poly (Butylene-Adipate-co-Terephthalate) (PBAT)

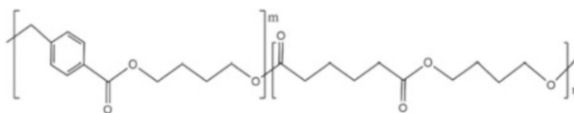
Poly (butylene-adipate-co-terephthalate) (PBAT) is a completely biodegradable synthetic biopolymer. PBAT is produced by poly-condensation of butanediol, adipic acid, and terephthalic acid from fossil fuel sources. The repeating unit of PBAT is shown in Scheme 15.

Along with being biocompatible, PBAT has excellent processing capabilities and flexibility. However, modifications are typically made to enhance its low strength and thermal stability. Contrary to other synthetic biopolymers, PBAT's flaws are significantly strengthened by introducing fibers, which boost their stiffness and thermal stability (Ferreira et al. 2019). However, using the centrifugal melt spinning

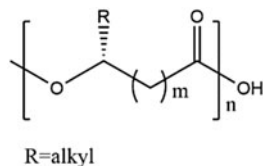
Scheme 14 Repeating unit of PBS



Scheme 15 Repeating unit of PBAT



Scheme 16 Repeating unit of PHAs



technique, the hydrophobic characteristics of PBAT may be modified to hydrophilic PBAT fibers (Li et al. 2021).

Poly(Hydroxyalkanoates) (PHAs)

Natural polyesters called polyhydroxyalkanoates (PHAs) are produced by various bacteria that use them as a source of carbon or energy. Several PHAs kinds have been reported with different monomers (repeating units) (Koller and Mukherjee 2022), as indicated in Scheme 16.

PHAs are entirely biodegradable and biocompatible. However, the thermo-mechanical characteristics of PHAs are significantly influenced by the chemical structure, co-monomeric composition, unit-comonomer composition distribution, molecular weight distribution, and average molecular weight due to the variability of its repeating units. Compared to other synthetic biopolymers, the main problem faced by PHAs is their high cost. Therefore, in addition to lowering PHAs' costs, PHA modification is also necessary to increase their functionality. The functionality of PHAs can be enhanced through various modifications, including chemical, curing, and surface modification. Chemical modification of PHAs such as chlorination, carboxylation, hydroxylation, and epoxidation successfully enhance the functionality of PHAs through the introduction of new reactive functional groups in polymer chains (Sharma et al. 2021). However, PHAs that have undergone chemical modification frequently yield materials with poor mechanical properties. Through curing techniques, either utilizing natural, chemical, or physical crosslinking processes, PHAs with good mechanical characteristics can be created (Jo et al. 2021). The hydrophilic characteristics of PHAs are improved through plasma surface modification, photografting, and surface polarization, particularly in biomedical applications.

Physical Modifications of Biopolymers

Plasma Treatment

In plasma treatment, the biopolymer is subjected to thermal treatment in an inert atmosphere that is enriched with reactive atomic, molecular, radical, or ionic species. The inert atmosphere can be gases like air, nitrogen, argon, oxygen, nitrous oxide,

carbon dioxide, water vapor, helium, ammonia, or methane. Plasma, “the fourth state of matter,” is a gaseous environment comprised of inert or reactive gaseous radicals, ions, polymers, monomers, etc. with an overall charge density of zero. Plasma produced between high voltage electrodes contains high energetic accelerated electrons. This high energetic plasma containing electrons or reactive ions hits the polymeric surface causing the hydrogen radical abstraction or covalent bond breaking leading to the further functionalization with the radical present in the reactive plasma atmosphere (Kale and Desai 2011). The collision of electrons with air particles might cause oxidation of the substrate surface. The reactive plasma atmosphere could act in two ways:

- (i) Bombard the surface causing a chain scission on the surface for etching or activation by non-polymerizing gases like Helium, Oxygen, Nitrogen, or Air.
- (ii) Plasma-induced grafting or polymerization involving polymerizing gases containing reactive precursors like hydrocarbons, silicon monomers, etc.

Plasma treatments cause surface modification rather than bulk modifications. The surface functionalization of the biopolymers depends on the nature of the polymer surface, pressure, gaseous atmosphere, etc. For example, the H_2O atmosphere causes the hydroxyl functionalization of the polymeric surfaces. The monomers for the surface functionalization can be of a reactive intermediate or any ring structures with some degree of unsaturation.

Plasma treatment of nanocellulose in air induced hydrophobicity whereas N_2/NH_3 induced hydrophilicity attributed to the surface functionalization by polar groups (Flynn et al. 2013). Contradictory to this, bacterial nanocellulose did not show any effect by air plasma treatment (Pertile et al. 2010). The effect of ozone and low-temperature plasma treatment and acetylation modification on cellulose in biocomposites has been discussed by Lenfeld et al. (2020). Chitosan can also be surface-modified hydrophilic using oxygen plasma treatment and UV/ozone treatment. Both the treatments produced the oxidized carbonyl group keeping the amino group and the hydroxyl group of chitosan intact (Matienzo and Winnacker 2002). Also, chitosan is made adhered over the cotton and polyethylene terephthalate (PET) fabrics via low-temperature air plasma treatment surface modification of cotton and PET. The formation of carboxyl groups increased the adherence of chitosan on those fabrics (Shin et al. 1998). Starch biopolymer has also shown an increase in hydrophilicity by plasma treatment with a contact angle between 5° and 14° (Wiacek 2015). PLA has also shown increased hydrophilicity using medium pressure plasma treatment (Jacobs et al. 2012) and the surface modification of PLA by air atmospheric plasma treatment has been discussed in a review by Vilaplana et al. (2014). Depending on the nature of the substrate, the plasma treatment can lead to cross-linking, ablation, pre-deposition, or surface activation by forming free radicals on the substrate surface itself. Plasma oxidation, amination, and nitration will increase the hydrophilicity of the biopolymer.

Plasma treatment can be performed in low temperature, low pressure, or as atmospheric pressure plasma jet (APPJ) or atmospheric glow discharge (AGD). AGD uses high-voltage high-frequency radio waves whereas APPJ developed by

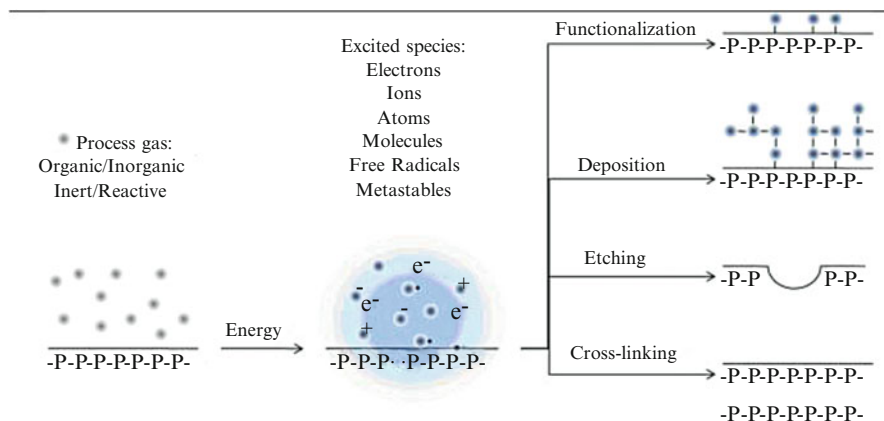


Fig. 2 Plasma treatment for surface functionalization. (Reprinted with permission from (Bazaka et al. 2011))

Jeong et al. (1998) uses atmospheric pressure plasma with temperature between 100 °C and 275 °C to treat a specific part of the biopolymer and also for three-dimensional structures and temperature-sensitive biological materials (Kalia et al. 2013). Low-pressure plasma uses atmospheric pressure or vacuum condition using low power output of about 60-100 W, thereby mitigating thermal damage to plant fibers and maintaining a uniform, high concentration of reacting species (Yuan et al. 2004). Plasma treatment is a clean, dry, and economically and environmentally benign technique with no emission of pollutants. It does not require any type of water or chemicals. It changes the hydrophilic and oleophilic properties of a biopolymer, wettability, and physical and electrical properties through surface modifications (Morent et al. 2008). Also, the plasma-treated biopolymers can be a platform for further surface modifications for the incorporation of different functionalities as per the need for application (Fig. 2) (Bazaka et al. 2011).

Plasma treatment might also pose some challenges like fragmentation due to the temperature conditions. Hence, a method of pulse plasma technique is now being used to give a fine control over the surface functionalization with good stability.

Bacterial Nanocellulose Coating

Coating with bacterial nanocellulose is a type of noncovalent modification. Non-covalent adhesion aids in physical adhesion or self-assembly of the macromolecules, polymers, nanoparticles, or any other modifying agents over the biopolymer surface. The method is very simple and sometimes later cured for stability (Choudhury et al. 2018). Bacterial nanocellulose stands different from other types of cellulose in terms of its high purity, water holding capacity, greater strength, and mold ability. The biopolymer plant fibers can be modified to increase the interfacial adhesion between the biopolymer and the matrix by just coating the fibers with bacterial nanocellulose.

The bacterial nanocellulose can be coated in situ by culturing the fibers in presence of bacterium like *A. xylinum* in an appropriate culture medium (Kalia et al. 2013).

Adsorption of Macromolecules

Macromolecules such as surfactants, copolymers, polyelectrolytes, or oligomers can be made adsorbed onto the surface of biopolymers via noncovalent interactions which tune the biopolymer for the hydrophobic properties. The adsorption on the biopolymer surface occurs via hydrogen bonding or intermolecular interaction or van der Waals forces (Habibi 2014). The surfactant molecules form a thin coating over the biopolymer which is evident from the small angle neutron scattering experiment studied on nanocellulose. Especially cationic surfactants like dodecyl trimethyl ammonium chloride possibly get easily adhered to the negatively charged surfaces of the biopolymer (Ansari et al. 2015). Uncharged macromolecules also adhere to the biopolymer surface. Thus the adsorption mechanism could tune the hydrophilic and hydrophobic properties and increase its dispersibility in a nonpolar medium (Kargarzadeh et al. 2018). The surfactant to biopolymer ratio greatly influences the hydrophobicity of the modified biopolymers. This type of interaction is not strong as the other covalent interactions and can easily be broken by vigorous stirring in excess water (Dhali et al. 2021).

Radiation

High energy gamma radiations, UV radiation, and even microwave radiations have also been applied for the surface modifications of biopolymers. The gamma radiations ionize the surface of the biopolymers creating the surface radicals or reactive ions which paves the way for further surface modifications like polymer grafting, crosslinking, oxidation, etc. for the tuning of hydrophilic or hydrophobic properties. Khan et al. reported the effect of gamma radiation on the water vapor permeability (WVP) of nanocellulose/methylcellulose composite film wherein WVP decreased from 41% to 28.8% (Salmieri et al. 2014; Kargarzadeh et al. 2018).

UV light has also been used for surface modification as a photochemical radical initiator for further surface modifications (Coulibaly et al. 2014). Microwave radiation creates an internal heat transfer within the biopolymer molecules which helps in further acetylation and hydrolysis which is reported with nanocellulose biopolymer (Kargarzadeh et al. 2018).

Chemical Modifications of Biopolymers

Mercerization

Mercerization is a technique used in the study of nanocellulose. It involves the use of concentrated NaOH solutions. Even though this is not a surface modification method, it involves a transition from cellulose I polymorph to cellulose II polymorph

which differs by the degree of crystallinity. Cellulose II is less crystalline than cellulose I and this difference will also be reflected in the hydrophobic and hydrophilic properties of cellulose. The transition of cellulose chains from parallel arrangement in Cellulose I to antiparallel arrangement in Cellulose II greatly affects the properties. This transition can be the effect of two reasons during this NaOH treatment: (a) disruption of the hydrogen bond network in the cellulose chain (Simon et al. 1988) and (b) the random presence of the anti-parallel and parallel chains in the junction of crystalline and the amorphous phase. Some papers report the combination of the two polymorphs in cellulose nanocrystals (Kobayashi et al. 2011; Kargarzadeh et al. 2018).

Esterification

Esterification reaction is a covalent modification involving the formation of ester bonds (-OCOR) to link modifying agents with biopolymers. Esterification occurs between hydroxyl group and acid or acid derivatives in acidic medium by the elimination of water. Many long-chain carboxylic acids or alcohols are reported to be reacted with the biopolymers to yield hydrophobicity in biopolymers. The biopolymer utilizes its hydroxyl group or modified carboxylic group to undergo this reaction.

For example, cellulose is converted to cellulose acetate via acetylation reaction with acetic anhydride. Catalysts like imidazole, trimethylamine, and lipase have been used to functionalize cellulose surface by the ester bond formation. Acid chlorides like dodecanoyl chloride and lauroyl chloride react with cellulose primary hydroxyl groups forming ester bonds by the elimination of HCl (Pasquini et al. 2008; Cunha et al. 2014). The long aliphatic chains thus incorporated can stabilize oil/water/oil interfaces (Kargarzadeh et al. 2018). Surface modification using anhydrides like succinic anhydride and maleic anhydride via simple coupling reaction with the surface hydroxyl groups of MFC is reported to produce negatively charged hydrophilic surface (Stenstad et al. 2007). The hydrophilic cellulose nanocrystals are made hydrophobic by a covalent interaction with octenyl succinic anhydride (OSA) for the application as a Pickering high-internal-phase emulsion (Chen et al. 2018).

Citric acid is used to tune hydrophilicity of lignocellulose via simple, cost-effective esterification, or etherification reactions. Cellulose undergoes esterification reaction whereas lignin undergoes etherification reactions (Fig. 3) (He et al. 2018). A simple solvent-free one-pot acid-catalyzed hydrolysis reaction and Fischer esterification reactions using natural di- and tri-carboxylic acids like citric acid, malonic acid, and malic acid are reported (Spinella et al. 2016).

Etherification

Etherification involves the formation of ether bonds (ROR) on the surface of biopolymers. The hydroxyl groups in the surface of biopolymers react with the modifying agent containing any best leaving group resulting in the formation of ether

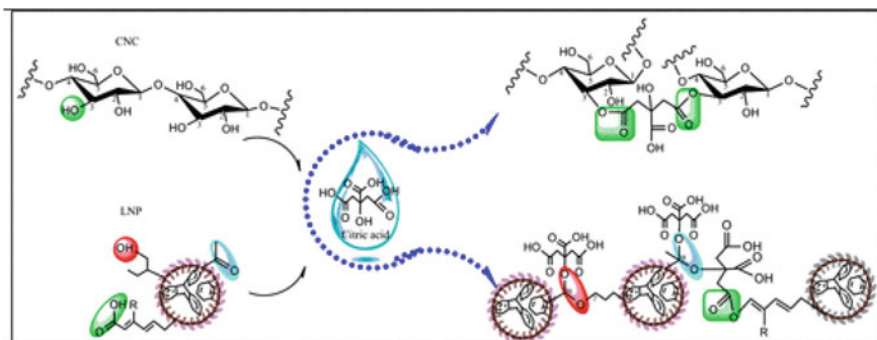


Fig. 3 Citric acid modification of cellulose and lignin. (Reprinted with permission from (He et al. 2018))

bond in an appropriate reaction medium. The long chain modifying agents undergo this type of reaction to increase the compatibility with a hydrophobic matrix. The application of this reaction comes mostly with nanocellulosic biopolymers. Long-chain octyl bromide or dodecyl bromide reacts with the cellulose to form hydrophobic nanocellulose derivative via ether bond formation. The bromide ion gets eliminated during etherification (Bae and Kim 2015). Hydrophobic anthraquinone derivative is also made to undergo the etherification reaction for the generation of hydrophobic nanocellulose material (Gorgieva et al. 2015). Also, hydrophilic cationic nanocellulose can be prepared by the etherification reaction between the hydroxyl groups of nanocellulose and the epoxy group of (2, 3-epoxypropyl)-trimethylammonium chloride (Hasani et al. 2008; Kargarzadeh et al. 2018).

Silylation

Silylation reaction is a type of covalent modification involving the reaction between the silane coupling agents and the nucleophilic surface groups like alcoholic, carboxylic, or amine groups of the biopolymer. A vast number of investigations have been reported with nanocellulose to produce the hydrophobic material with a decrease in the interfacial tension between the polymer and a hydrophobic matrix or organic solvents. Cellulose could be modified using organofunctional silanes to form Si-O-cellulose and Si-O-Si bonds to induce hydrophobicity via condensation reaction (Abdelmouleh et al. 2004). Organofunctional silanes like triethoxyvinylsilane, n-butyldimethylchlorosilane, n-dodecyldimethylchlorosilane, octyldimethylchlorosilane, 3-aminopropyltriethoxysilane, isopropyldimethylchlorosilane, and hexamethyldisilazane have been used for the silylation of nanocellulose (Dhali et al. 2021). If the degree of silylation is not properly controlled, the crystallinity of the biopolymer will be lost, hence proper control of reaction needs much attention. Photolithography can also be used to deposit hydrophobic alkylsilane layer over CNC via chemisorption. Photolithography can also be used to deposit hydrophobic alkylsilane layer over CNC

via chemisorption (Kargarzadeh et al. 2018). The Kenaf nanocellulose fiber-carrageenan macroalgae biopolymer film is made hydrophobic by modification with trimethoxysilane in methanol (Oyekanmi et al. 2021).

Silanetriols in neutral aqueous solution had been demonstrated as a silane modifying agent by Spirk et al., generating a hydrophobic material. This exploited the surface hydroxyl groups of the biopolymers essentially polysaccharides. Later, the oxidation of these hydrophobic surfaces using ozone/UV could generate hydrophilic compartments on these biopolymer substrates. Hence, a combination of these two techniques could finely tune the surface properties of a biopolymer (Spirk et al. 2010).

Silane coupling agents like 3-glycidoxypolytrimethoxysilane, 3-aminopropyltriethoxysilane, and a Titanate coupling agent are used to fabricate a hydrophilic microfibrillated cellulose (Lu et al. 2008).

Polymer Grafting

Polymer grafting involves the covalent bond formation between long-chain aliphatic polymers and reactive sites in the surface of the biopolymers to improve its hydrophobic properties. The polymer grafting can be a successor of the other modifying techniques like esterification, etherification, silylation, acetylation, plasma, or radiation so as to generate reactive sites on the surface of the biopolymers. There are two grafting techniques involved in polymer grafting: “Grafting from” and “Grafting to” approach. “Grafting from” approach involves the growth of the polymer right from the reactive sites present in the biopolymer whereas the “Grafting to” approach is the technique of grafting the already designed polymer chain onto the surface of the biopolymer via any covalent reactions. “Grafting from” approach can result in the design of the higher density polymer grafted biopolymers due to less steric hindrance whereas the “Grafting to” approach results in low-density polymer grafts due to more steric hindrance of the already grafted polymer. “Grafting from” approach employs atom transfer radical polymerization or ring opening polymerization and “Grafting to” employs click chemistry approach or epoxy groups or nucleophilic addition (Kargarzadeh et al. 2018; Dhali et al. 2021). Other grafting techniques involve chemical vapor deposition (CVD), plasma enzymatic, etc. (Choudhury et al. 2018).

TEMPO-Oxidation

TEMPO (2,2,6,6-tetramethylpiperidine-1-oxyl) radical oxidation of biopolymers involves the hydrophilic modification of the biopolymers by the oxidation of primary hydroxyl to carboxylic acid groups. The dense polar carboxylic acid groups increase the dispersibility of the biopolymer in aqueous media. The negative surface charge density on the surface of the biopolymer increases its hydrophilicity. Also, these carboxylic acid groups can be a gateway for the other modifications for the biopolymers. In the case of cellulose, primary hydroxyl groups at C6 carbon atom undergo TEMPO oxidation (Kargarzadeh et al. 2018; Dhali et al. 2021).

Acetylation

Acetylation reaction aids in increasing the hydrophobicity of the biopolymer by the treatment with a high concentration of acetic anhydride in presence of a catalyst via a simple, cost-effective procedure. The acetate ester groups at the surface groups of biopolymer increase the dispersion of the biopolymer in nonpolar hydrophobic matrices. Acetylation also acts on buried hydroxyl groups of cellulose nanocrystals in addition to the surface hydroxyl groups. Single route to the acetylation procedure has been reported by Cetin et al. via trans-esterification reaction with vinyl acetate (Çetin et al. 2009).

Sulfonation

Sulfonation helps in the production of biopolymers with enhanced hydrophilicity and good dispersion in aqueous media. The presence of surface hydroxyl groups in the biopolymer undergoes hydrolysis with sulfuric acid to form sulfate half ester moieties. These half-ester moieties are negatively charged which enhances its dispersion in an aqueous medium by inter-anionic repulsion between the biopolymer particles. But sulfonation alone could not produce high density of sulfate ester moieties; hence an oxidation step must also be needed after sulfonation step. Chlorosulfonic acid has been used to modify nanocellulose by sulfonation reaction which showed a large negative surface charge density (Luo et al. 2018; Dhali et al. 2021).

Epoxidation

Another modifying technique includes epoxidation. Stenstad et al. reported the epoxidation of microfibrillated cellulose via oxidation with Cerium (IV) generating free radicals followed by grafting by Glycidyl methacrylate (Stenstad et al. 2007).

Other Covalent Modifications

Amidation

Grafting of hexamethylene diisocyanate followed by amination introduces positive charge on the surface of MFC. The grafting of hexamethylene diisocyanate gives hydrophobic nature but the excess isocyanate introduces allophanate groups with free amino groups which makes the surface hydrophilic (Stenstad et al. 2007).

Modification with Natural Polyphenols

The hydrophobic nanocrystalline cellulose is fabricated from micro-crystalline via oxidation-citric acid esterification-octadecylamine amidation (Majdoub et al. 2021).

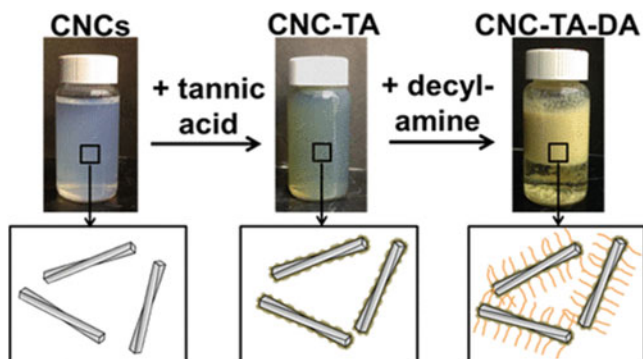


Fig. 4 Covalent Mannich modification of cellulose nanocrystals with tannic acid and decylamine. (Reprinted with permission from (Hu et al. 2017))

The nanocellulose can be covalently modified with decylamine via a Mannich reaction forming a hydrophobic tail part over the nanocellulose nanocrystals with the involvement of tannic acid as a primer for the modifications in water media (Fig. 4) (Hu et al. 2017).

Modifications by Enzymes and Amino Acids

Starch is reported to be modified with lysosome solution which increased the hydrophilicity and antibacterial property (Wiacek 2015). Further, polyamide was surface modified with 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA) for anti-fouling property by enhancing the acid-base interaction between the fouling agent and the polyamide reverse osmosis membrane (Azari et al. 2014).

Modification with Chitosan

Chitosan is deacetylated derivative of chitin, the natural polycationic linear biopolymer derived from the exoskeleton of insects, crustaceans, and cell wall of fungi. The structure of chitosan consists of randomly arranged β -(1–4)-linked D-glucosamine and N-acetyl-D-glucosamine randomly distributed within the polymer. Chitosan modifies the other biopolymers to become more hydrophobic like it modifies the PVA, a synthetic biopolymer imparting them with enhanced hydrophobicity (Jayasekara et al. 2004). Starch is also reported to be modified with chitosan solution and the contact angle measurement showed an increase in the hydrophobicity of the biopolymer and antimicrobial property (Wiacek 2015). Chitosan is reported to be linked with natural clay mineral, Halloysite exploiting the amino and hydroxyl functional groups of chitosan and the hydroxyl groups of the mineral, thereby tuning the hydrophobicity of the hybrid material (Tan et al. 2016).

Triazine Modification

Triazine chemistry for the modification of nanocellulose was carried out using octadecyl, benzyl, propargyl, and monoallyl-PEG triazinyl derivatives via aromatic nucleophilic substitution in presence of a base. The surface-modified nanocellulose showed hydrophobic or hydrophilic nature as per the modifying Triazine (Fatona et al. 2018).

Conclusion

Biopolymers are a class of polymers that are either biodegradable or derived from natural resources or both. Biopolymers are attracting marked attention due to environmental concerns and the growing need to decrease dependence on fossil and petroleum resources. Biopolymers possess many unique properties which make them potential alternatives to petroleum-based polymers, such as renewability, biodegradability, nontoxicity, biocompatibility, being environmentally friendly, etc. They also have mechanical properties comparable to conventional polymers. Due to the presence of reactive groups, biopolymers exhibit a wide degree of functionalities that allow physical and chemical properties to be modulated for intended applications. Surface modification of biopolymers is a well-known technique of functionalizing biopolymers to new materials with superior properties and applications. The physio-chemical properties of biopolymers such as hydrophilicity/hydrophobicity, water or organic solvent solubility/dissolubility, mechanical, thermal, and rheological, properties, etc. can be modified via chemical or physical surface treatments. When selecting a chemical modification, it is important to consider the simplicity of the chemical modification process. The complex modification process may hinder production scale-up. The selection of an appropriate modifying agent, and modifying technique must be done carefully in order not to weaken other intrinsic properties of the biopolymer such as its biodegradability. While some of the modification processes have been successfully implemented on a large scale, there is a growing concern about the environmental impact of these processes, increasing the need for the eco-friendly modification techniques and green modifying agents.

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Part V

Biopolymer-Based Blends, IPNs, Gels, and Composites



Sanjiv Sonkaria, Ji-hyun Cho, Hyeon Su Jo, and Hyun-Joong Kim

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Abstract

The indispensable nature of plastic-based materials in packaging processes and their widespread global dependency marks an era of a “plastic crisis” with toxicological and environmental consequences to all living entities in our ecosystem. The potential biohazards associated with plastic manufacturing industries resulting from the chemical breakdown to toxic components present a challenging technological issue. While the petroleum-based plastic market is predicted to shrink marked by a notable effort toward an emerging bioplastic market bearing a low environmental load, the shift is expected to abolish the dependency on plastic

S. Sonkaria · J.-h. Cho · H. S. Jo

Laboratory of Adhesion & Bio-Composites, Program in Environmental Materials Science, Seoul National University, Seoul, Republic of Korea

e-mail: ssonkaria64@snu.ac.kr; chyun1029@snu.ac.kr; whgustn110@snu.ac.kr

H.-J. Kim (✉)

Smart Center for Adhesion Research & Development (Smart CARD), Laboratory of Adhesion & Bio-Composites, Program in Environmental Materials Science, College of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea

e-mail: hjokim@snu.ac.kr

use in a plastic-free society. The bioplastic growth trajectory is discretely rising, but advancements have been dampened by price economics and the underperformance of biobased plastics due to material properties in comparison to their counterparts. Polymer blending is considered an important route in the design of new materials' properties to incorporate adhesive and interfacial features to impart biodegradable characteristics in the form of bioplastics. This chapter aims to present various approaches to blending strategies and to discuss the physical and chemical limitations of polymer blending, and structure-property relationships can yield bioplastics as viable materials.

Keywords

Bioplastics · Polymer blends · Copolymerization · Biodegradability · Biopolymers · Microbial degradation

Introduction

Polymers are the most abundant commercially used materials. Consumer-driven use of plastic use has exponentially increased. Alarming statistics reveal that one million plastic bottles reach consumers every minute and five trillion plastic bags are in circulatory use each year (United Nations, Environment Programme 2022) – a situation that promises to worsen with devastating consequences with the rise of industries (Mecking 2021). Plastic materials by their compositional nature are “single use products” and can show considerable resistance to degradation under ambient conditions making them heat, force (e.g., mechanical) and chemically, moisture, biologically, and weather resistant. This resistance can span decades of slow toxic release of plastic constituent materials into the environment. The consequential ripple effect of nondegradable plastics is driven by the dynamics of mother nature in the form of high velocity winds and ocean currents from local to global scales spreading to oceans (Jenna Jambeck et al. 2015) resulting in their accumulation (Lebreton et al. 2018) in ecosystems as far as the Arctic polar regions on the planet (Bergmann et al. 2022). The disintegration of plastics into secondary products in the form of microplastics (Zhang et al. 2021) from their bulk counterparts which exist on the millimeter scale or smaller is environmentally catastrophic to marine, land, and human life and results from the lack of reliable methods to detect and remove small-scale contaminants particularly if embedded within animals that enter the human food chain. Consideration is now being given to the fate of nanoplastics that find their way into reproductive organs of organisms which can be inherited by their offspring (Zhao et al. 2017). Plastic waste has now reached catastrophic proportions threatening the existence of life incubated by an exponentially increasing microtoxic environment.

The purpose-built nature of plastics as long durable polymer chains is resistant to environmental stress and strain, chemical attack, and physical absorption which are variable features of different plastic compositions. Such characteristics define material toughness, stiffness, mechanical properties, moisture and gaseous absorption (vapor diffusion), hydrolysis, compressibility (mechanical load) and creep, and UV

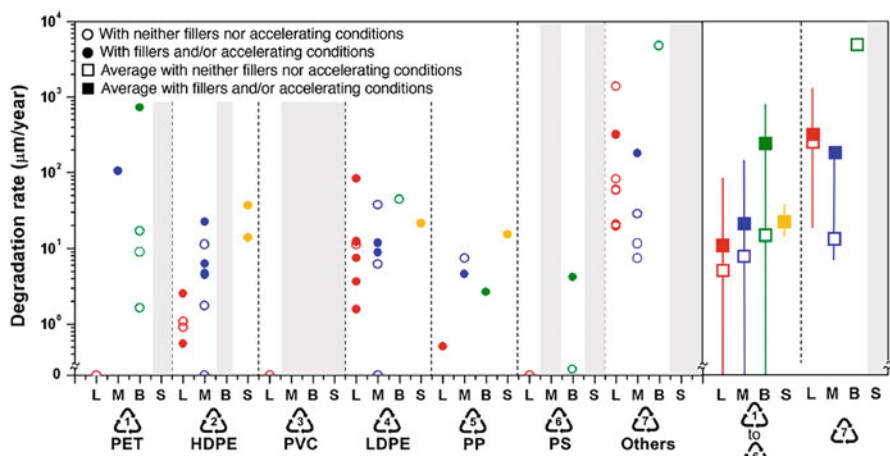


Fig. 1 Comparison of plastic degradation rates of Landfill (L), Soil (S), Marine (M), Biological (B), and Sunlight (S). (Reprinted from (Chamas et al. 2020). This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0))

resistance. Questions that are universal to understanding the physical and chemical resistance of plastics and many pertinent issues related to plastic degradation remain elusive (Chamas et al. 2020). The variability in degradation rates of commonly categorized plastics summarized in Fig. 1 provides insightful clues of the persistence of polymers in the environment, and their degradation profiles can be highly multiphasic as the disintegration of the materials depend on their dimensional occurrence, polymer type and complexity, filler content, and susceptibility to degradation and the surroundings. Such factors decidedly determine the fate of degradation that may be mediated by chemical, physical, or biological means.

Reducing plastic waste consumption through the utilization of alternative material packing and increasing efforts to recycle plastics can certainly slow down the impact of plastics and their derivatives. However, the huge global dependency on plastics from food containment to pharmaceutical packaging inevitably means that the demand for cheap plastics will continue to contribute to this growing crisis. Hence, there are growing concerns that recyclability which applies to only 10–25% of waste plastics will not be able to sustain and accelerate growth to a plastic-free environment. To overcome this limitation, it is essential to ensure that plastic utilization is aimed to be entirely recyclable or to undergo some process to reinvent waste plastic into a reusable form. Solutions to resolving the sustainability of environmentally friendly and degradable plastics have become an urgent matter of formulating polymer chemistry through engineering of molecular building blocks and bond accessibility for safe degradation using a multidisciplinary approach involving aspects of polymer chemistry, materials engineering, and biological science (Fig. 2). This reflects that the future of plastic formulation has evolved into a multifaceted cross-disciplinary area (Belontz et al. 2019). This will likely involve engineering new functionalities into plastics for the facile disassembly of persistently long-chain lengths into smaller components which are not toxic (Yun 2011).

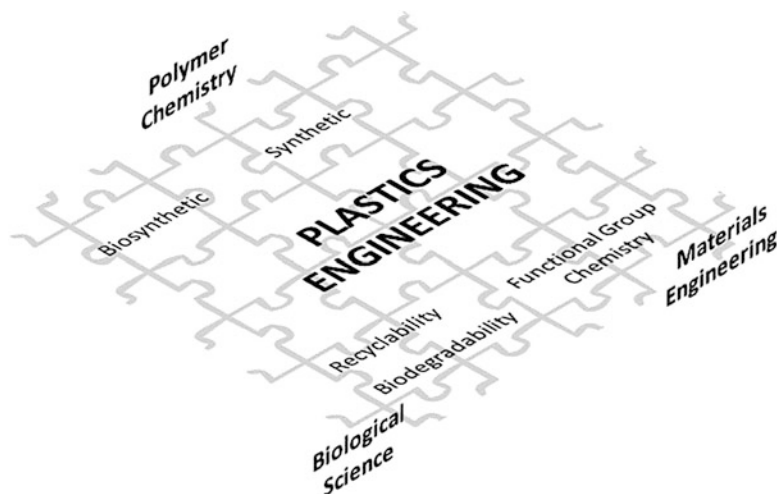


Fig. 2 Multidisciplinary overlap to engineering plastics

Breaking of C-C and C-H bonds via oxidative bond cleavage resulting in the fragmentation of the carbon framework into removable or nontoxic components on a timescale that can process tonnes of quantities of plastic on a daily basis is currently unachievable. A key goal is to deliver a rational technology to modify the polymer skeleton of plastics by weakening bonded structures and making available new entities for transformations into precursors for manufacturing of industrially important constituents (Vollmer et al. 2020). Ceasing such opportunities for the conversion of degraded synthetic by-products into commercially useful avenues can visibly have a positive impact on the environment.

Mechanisms that involve bioremediated destruction of polymers by microorganisms are inspirational by design provided by nature itself. Bioplastics from a cost perspective are considered expensive, but their interest has slowly but steadily increased over the last decade. A hallmark of biodegradation is often signified by metabolic products – carbon dioxide, water, and methane – driven by electron transfer processes that utilize oxygen, hydrogen, and metals and thus facilitating bond cleavage for energetically demanding bond conversions.

A promising route that has steadily gained considerable interest over the last few decades is the concept of polymer blending. Principally, polymer blending is based on the rationale of tuning the physical and chemical characteristics of polymers which when combined into a single polymer, certain properties can be suppressed or enhanced, or more probable is the introduction of new characteristics acquired by the resulting blend. Polymer blending has the potential to deliver renewable plastics as new materials from existing sources through mixing and processability by overcoming barriers to their formation that may be chemically demanding. There is an unmet need that combines the economics of material production with selected engineered properties that falls within the periphery of stable plastics for the purpose they are

made but adequately unstable for less costly degradation strategies. These limitations can be overpowered by the design of blended polymers which can structure and shape materials for one of the most sought after functionality of materials today – controlled degradability of by-products.

This chapter brings a recent perspective on polymer blending strategies to introduce new material capabilities as viable solutions to ultimately reduce the environmental burden from the overload of plastic released into the far-reaching habitats of our ecosystem and beyond through ocean waste (Suaria et al. 2016; Jambeck et al. 2015). We also discuss the limitations of polymer blends with a view to explore some of the key reasons that must be addressed in order to secure the future from the growing threat of plastic intoxication.

Nonbiodegradable Degradation of Plastics (Abiotic)

The chemical degradation of polymers that do not involve the action of microbial components in their breakdown to liberate polymer fragments is termed as abiotic. Early investigations on biopolymers, namely, starch, show that they ordinarily degrade assisted by the presence of prooxidant (Koutny et al. 2006) which under abiotic conditions driven by thermal aging degenerates to monomer products released as ketones, aldehydes, alcohols, lactones, and carboxylic acids (Albertsson et al. 1994). Polymers receptive to photooxidation also represent important abiotic pathways (Fig. 3) through which common plastics can absorb external light energy to excited chemical states and photo-degenerate to smaller stable products (El-hiti et al. 2022). Under changing weathering conditions, synthetic plastic/polymer degradation is unfeasibly slow spanning decades with very limited depolymerized fragmented product evolution. Evidence has recently surfaced which points to the abiotic chemical destruction of potent chlorinated tetrachloroethene (PCE) and trichloroethene (TCE) using abiotic processes. An abiotic route for the accelerated breakdown of synthetic plastics was shown by the addition of oxo-additives to synthetic plastics PP, namely, oxo-degradable polypropylene (PP-oxo) and high

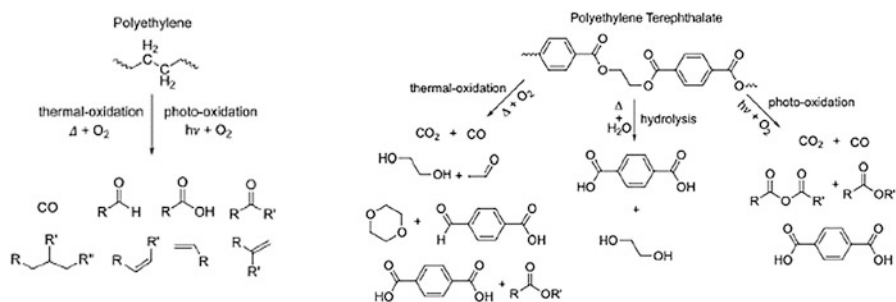


Fig. 3 Degradation products generated from abiotic products. (Reprinted from (Chamas et al. 2020). This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0))

density polyethylene (HDPE-oxo) under weathering differing conditions (Ángeles-López et al. 2016). The study reveals that it is possible to reduce plastic resistance to disassemble under ambient conditions. Further, the use of Fe(II) at concentrations of 20 mM were effective in the structural degradation of chlorinated polymers resulting in the release of detectable PCE and TCE reduction products (Entwistle et al. 2019).

A recent study attempted to decipher some of the complexities associated with plastic degradation by probing abiotic and biotic activities related to polymer degradability and structure-property data as predictors for screening underlying hierarchical patterns of variable polymer types. Using a key parameter defined by hydrophobicity ($\text{Log}P$) modeling, crystallinity, T_g , number-average molecular weight (M_n), and dispersity (M_w) were screened against 110 polymers exhibiting a wide range of hydrophobicities (Fig. 4b) using a computational predictor tool, modeling (Fig. 4a), and machine learning. The findings provide significant insight of functional group chemistry of carbonates, esters, and amides with low hydrophobicity that assists abiotic hydrolysis and biotic mechanisms. This is in contrast to higher $\text{Log}P$ values correlating to C-H bond the breakdown of which is perturbed by additives. Polyester hydrolysis is largely influenced by positive changes in values of $\text{Log}P$ and crystallinity affecting degradability. Further, the study concludes that biotic mechanisms operate more rapidly compared to abiotic routes (Min et al. 2020).

The Problem with Plastic and the “One-Way” Transition from Recycling to Polymer Blends

The long-chain composition of polymers and the resulting complexity in terms of shape and structure are usually a result of strong bonded networks intricately defined by assembly patterns of repeated units locked into a predetermined configuration. Thus, molecular bond chemistry is at the heart of the “plastic” industry. Decades on, polymer chemists are now working to undo what their predecessors have worked tirelessly to achieve – strong polymer plastics. However, reversing tough polymer bond synthesis is no easy task. While some plastics are easily reversible, others pose a huge environmental burden. The variability in polymeric bond strength, the toxicity of breakdown products, and the recovery of starting materials coupled to the cost and severity of processes pose workable barriers to manage plastic disposal. While it is chemically feasible to drive polymerization reactions from monomeric units, it is energetically demanding to depolymerize some of the more common plastics. For instance, polyolefins composed of polymerized alkenes are an example of some of the most difficult plastics to degrade and are estimated to decompose only up to 0.5% of their original structures over a period of 100 years. Polyolefins such as polypropylene and polyethylene are ubiquitous industrialized plastics used for their high density, low density, and linear low-density characteristics with wide-scale properties highly resistant to oxidation in their hydrophobic states (Koteswara Reddy and Kiran 2019).

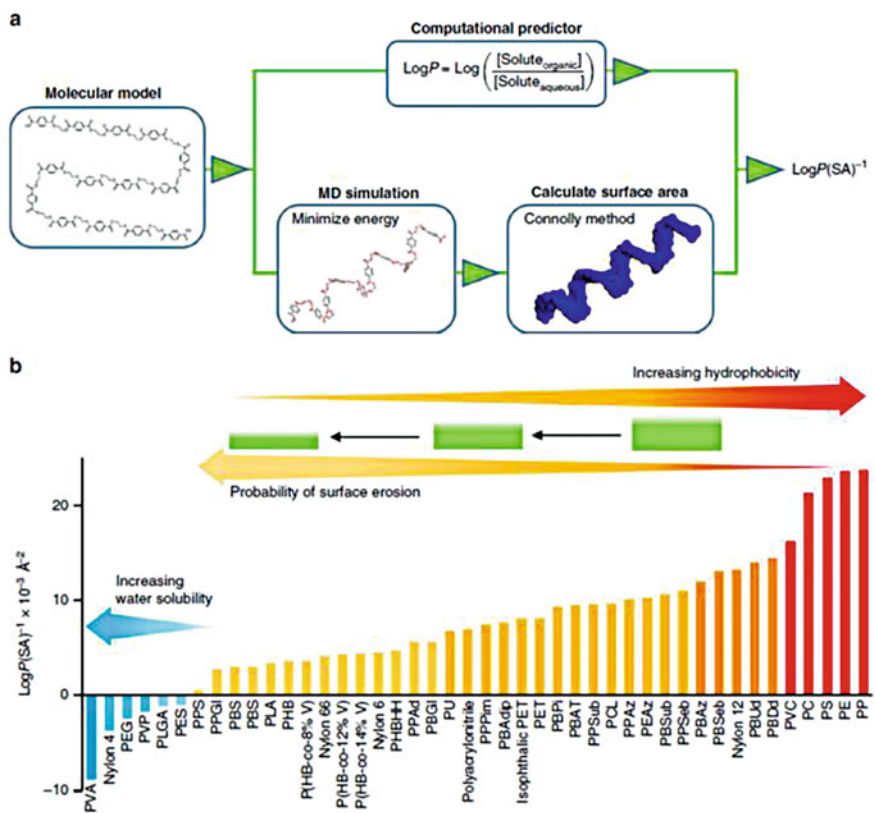


Fig. 4 Functional group chemistry of abiotic and biotic activities revealed by (a) predictor tools used in machine learning for (b) profiling polymer hydrophobicity and water solubility. Reprinted from (Min et al. 2020). This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0)

Due to bond strength, pyrolysis continues to be a commonly chosen method for breaking polymers into smaller units. With temperature, time, and pressure being key parameters for steering the molecular assembly of polymers, the same parameters are being used to control the release of monomers from their polymeric counterparts as a pyrolysis-based renewable technology for recycling complex plastics on a commercial scale. The technology drives the process to deliver useable monomers from a high carbon feed to a low carbon output by minimizing the oxygen availability of the environment. Mundane degradation caused by weathering conditions in the natural environment typified by low susceptibility to polymer oxidation and hydrolysis' subsequent breakdown is both uncontrolled and impractical for managing plastic pollution. The initial conversion of hard-to-degrade plastics from a state of low-to-high susceptibility for bond cleavage to occur requires (1) processes which can match or exceed the current and future rate of plastic utilization; (2) the

conversion or incorporation of bond types that are accessible to bond fission; and the (3) controlled release and containment of degradation products for safe disposal, particularly microparticles that often find their way into the digestive tracks of animals.

While toughness has been a key attribute to polymer performance, environmental policies are reshaping a new trend in polymer design, and time and cost are an added advantage of bond making and bond breaking. Although high-temperature recycling and polymer blending approaches are conceptually different, both methods border on the same on delivering low-carbon products as precursors for further recycling and commercialization (Fig. 4). However, the primary advantage of using polymer blending to pyrolysis or catalytic processes for disposing chemicals is the suppression of volatile toxic fume release such as chlorine from poly(vinyl chloride) which is often uncontrolled in the latter process. Gaseous by-products like hydrochloride render heat-driven processes unsuitable, and polymer blending using chemical approaches could be a more environmentally acceptable procedure to possibly reduce, divert, or even eliminate poisonous side products during the monomerization of polymer chains (Walker et al. 2020). Using the example of polyolefins as one of the most persistent man-made plastics that exist on our planet, processes which involve oxidative degradation usually via the absorption of thermal energy from natural sunlight result in excessively slow degradation (0.5%) of the polymer that spans decades. The O_2 activation from the sun's energy can directly permit bond scission of the carbon chain and liberate free radical structures with variable energies, and the radicals formed hold the potential to introduce a new array of reactive moieties such as carboxylate and hydroxide anions and hydrocarbon radicals which pacify other radicals to form stable shorter chains and molecules (Fig. 5). The introduction of localized hydrophilic regions within the hydrophobic polymer through high energy absorption of thermal energy from the surroundings forming smaller structures can be further used by microorganisms as a source for carbon. The utilization of carbon units in metabolic product assembly is coupled to CO_2 and H_2O release and underlines processes that essentially support life. This can only occur by the interaction of microbial cells with recognizable structures and their entry into cells. However, most plastics have almost negligible oxodegradation profiles and must be assisted by additional groups that are aligned to nature. Considerable efforts are being applied to engineer nonbiodegradable plastics derived from petrochemicals into biodegradable forms to enable their interaction and uptake by microorganisms for carbon recycling. Polymer blending is one such process to formulate new but more desirable properties by combining existing ones which is inherently different to the intrinsic nature of the separated polymer components. Hence, copolymerization of the blended components offers a cheaper alternative to modify polymer properties using a facile route compared to the polymerization of new monomers or their reformation prior to polymerization.

Polymer blending as an approach to tailor polymer properties as desired for task-specific functions has gained superiority to using base materials for the assembly of different types of plastics with variable characteristics that traditionally depended primarily on synthetic conditions. The distinctive functional features of the new

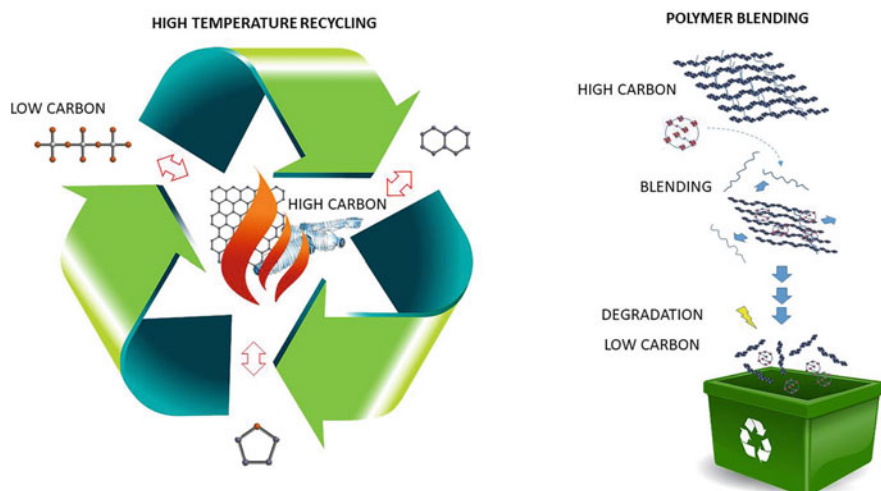


Fig. 5 Thermocatalytic recycling of complex plastics and polymer blending of synthetic polymers leading to the introduction of engineered properties for facile degradation into useful recyclable precursor products

polymer blend take advantage of the most economical and most versatile approach to access the most diverse property range completely different from its constituent properties. In view of the current accumulation rates of plastic (Geyer et al. 2017) and its effect on the natural environment, the complete recovery of carbon from plastic for renewable use is a leading mandate of the twenty-first century. Degradation strategies are now geared toward biobased plastics composed of compostable and biodegradable polymer frameworks. A central concern to the design of biodegradable plastics is the degree of synergistic interaction and compatibility during coprocessing of the blended components to make the process and the product as feasible as possible. While the properties are different to the source, both the physical and chemical properties are dependent on individual elements that come together in a single phase rather than as a composite in which the components are separated by virtue of their own properties in a multiphase. Structural factors such as complexity, bond type, composition, polymer arrangement, and the degree of crystalline / amorphous morphology and physical properties like solubility, density, and molecular weight and toughness ultimately determine the polymer degradability (Zeenat et al. 2021).

Biodegradable Plastics: Understanding Microbial Interaction

To gain an appreciable understanding of the mechanisms microbes use in their interaction and degradation of plastic, it has become necessary to profile the inherent structural and functional characteristics microbes use to derive carbon from plastics

waste. Modeling microbes versed in polymer chain scission can provide a potential wealth of information in understanding the mechanistic aspects of biodegradation of polymers to useful precursor materials such as hydrocarbons (Pundhir and Gagneja 2016). In the same context, biostability of plastics that show resistance to degradation in biological environments is equally of interest to determine how material persistence is related to functional group chemistry (Padsalgikar 2017). Some earlier observations demonstrated the biodegradable activity of microbes toward synthetic plastics. The parametric of weight loss, polymer elongated shape change, and tensile strength were notable effects of the interaction of lignose microorganisms with heat and UV-treated oxidized polyethylenes under culture conditions (Lee et al. 1991). Microbial degradation of UV-irradiated samples showed superior performance indicating increased susceptibility to the UV-induced oxidized state of the polymer and hence better accessibility to bond cleavage and highlighting the importance of the pro-oxidized weakened state of the extensive carbon skeleton framework. This is suggestive of the notion that a thermo-or UV oxidative pathway is the preferred route by which oxygen can liberate shorter chain lengths from polymers that are elevated to structures of higher energetic order. This can initiate chain scission events along the length of the polymer resulting in radical formation (George Wypych 2015) (Fig. 6). The diverse range of detectable oxidative products that include a wide selection of functional organic structures including alkanes, esters, alcohols, carboxylic acids, aldehydes, ketones, and keto acids, among others, brings into question the multiple mechanisms nature microorganisms employ to access (Hakkarainen and Albertsson 2005).

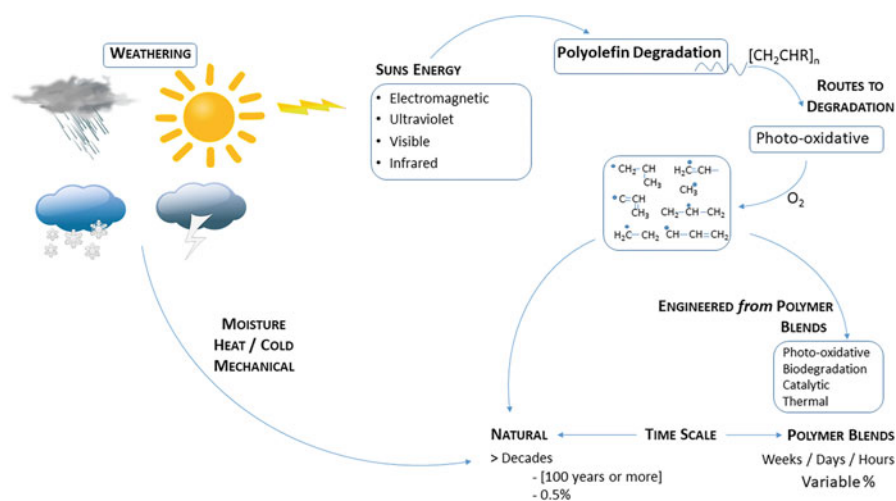


Fig. 6 Slow polyolefin bond degradation under natural weathering conditions can be accelerated by the introduction of engineered polymer blends

Barriers to Biodegradation

Some of the common commercially used plastics are shown in Fig. 7. Most of the polymers targeted for degradation by microbes are naturally occurring polycaprolactone (PCL) and polylactic acid (PLA) and have not evolved to efficiently disassemble unsaturated bonds of the C = O and C-O-C type which are often further stabilized by additives as plasticizers or fillers as supports (Björkner 2000). However, there is considerable debate surrounding the biodegradation of synthetic of plastics by microorganisms (Lear et al. 2022) and their standardization in general to degradation (Chamas et al. 2020). Modest efficiencies typically less than the 20% upper limit which falls far below the expected threshold required to diminish the plastic population to nontoxic levels. Currently, the high molecular weight of macroscale plastics and the addition of fillers, plasticizers, and antioxidants intensified to their general inertness make accessibility to these chemical functional groups extremely difficult as microbial substrates. Other barriers to biodegradation are shown in Fig. 8. Degradability of high resistant plastics notably low density polyethylene (LDPE), high density polyethylene (HDPE), polypropylene(PP), polyvinylchloride (PVC), polyethyleneterephthalate (PET), and polystyrene (PS) is largely attributed to high bond strengths which usually require temperatures

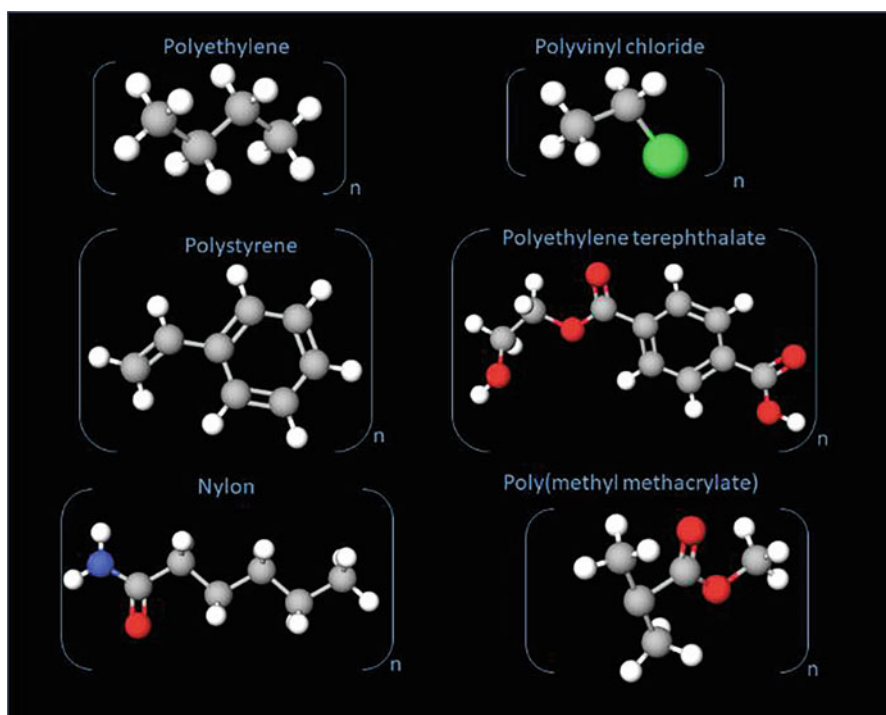


Fig. 7 Structures of some common plastics used in commercial products in a varying proportion

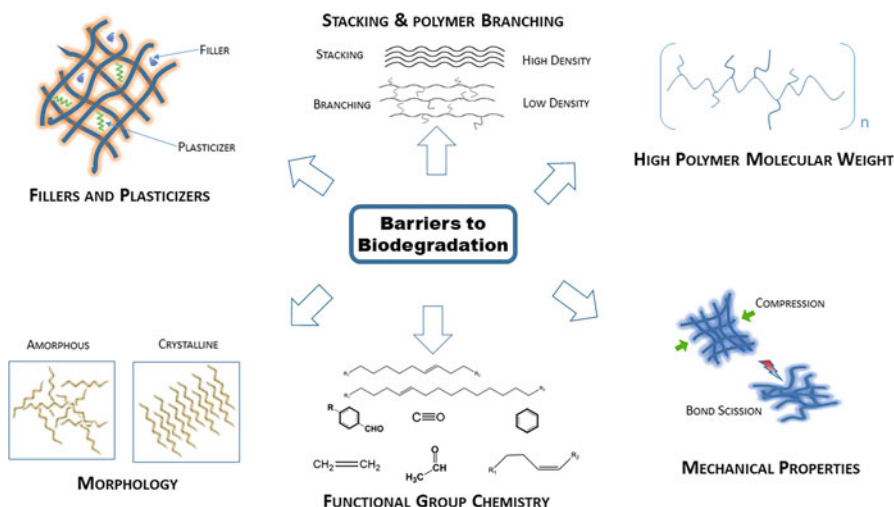


Fig. 8 A summary diagram of the barriers to biodegradation through natural processes

exceeding 100 °C and external mechanical stresses ranging from 10 to 15 MPa to break them. The alignment of the polymer bond is enhanced through the stacking of chains which contrasts the arrangement of branched polymer morphologies forming less densely packed structures which are more accessible to bond scission and enzyme degradation. Unrelated to the stacking or nonstacking nature of the polymer, the morphological state of plastics that reside between the fully crystalline and amorphous state impacts the extent to which it can be biologically degraded (Ronkvist et al. 2009) suggesting that microbial enzymes such as PETase may be better suited to depolymerize plastic materials of semicrystalline character and aromatic polyesters while showing little to no specificity for aliphatic structures. Also functional group chemistry exhibited by plastics becomes important not only for the potential for degradation but also for the utilization of degradation products as a carbon source for energy and metabolic product growth as demonstrated by the assimilated use of poly(ethylene terephthalate) (Yang et al. 2016). Perhaps the biggest barrier to biodegradation is functional group chemistry. Since nonpolar aliphatic C-C bonds are intrinsically inert, their uncreativity renders them demanding to split enthalpically. The exceptional stability of the carbon bonds makes it difficult to excite bond opening to more stable complexes particularly those of the metal type via an insertion mechanism (Jun 2004). However, C-C bond cleavage is ubiquitous in nature, and the high bond inertness necessarily requires access to functionalized groups within the polymer structure that is integral to biological catalysis. As in enzymatic cleavage of C-C bonds, transformation to other forms requires redox reactions which is often not the case with C-N and C-O bonds in the case of cytochrome P450 reactions (Guengerich and Yoshimoto 2018). Hence, bacterial enzymes can target polymer degradation products such as aldehydes and ketones for conversion to alkenes, alcohols, and acids from aromatic

hydrocarbons via carbocation and de-saturated intermediates, electrophilic, decarboxylation, nucleophilic, desaturation, and oxidative cleavage-driven reactions.

Hence, in the same context, it becomes crucial to understand the importance of functionalization of synthetic polymers in plastics in pursuit of their biodegradation. Plastics that use polyvinyl alcohol (PVC) and polyethylene succinate (PES) are able to accommodate more cleavable groups in their structure in the form of hydroxyl and ester moieties, respectively, which are recognizable by a large number of microbial strains. The incorporation of hydroxyl groups in highly resistant polymers can considerably aid polymer breakdown as modeled by polyethylene glycols (PEG) containing terminal hydroxyl groups (Rogers et al. 2019), and the conjugation of PEG-OH can provide added hetro-functionality to biopolymers like chitosan broadening their utility (Wu et al. 2008). Recently, Hadad et al. (2005) found carbonyl residues induced through photo-oxidation had a strong correlation with biodegradation activity of polyethylene via the thermophilic bacterium *Brevibaccillus borstelensis*.

Polystyrene, on the other hand, is strongly hydrophobic, variably thick, and a very durable plastic which makes the polystyrene composition hard to degrade in most landfill and soiled environments. The chemical modification of the polystyrene is required for microorganisms to adapt for the metabolic feed to occur usually oxidative enzymes to make available metabolically utilizable carbon-based substrates. This can occur readily under UV irradiation (Yousif and Haddad 2013) which initiates the production of mobile radicals that recombine following carbon-carbon bond chain scission to form energy precursors (Fig. 8). In the absence of a high energy input, PS degrading bacteria isolated from the gut of superworms have been identified as a serine hydrolase where the enzyme activity is characterized by a shift from hydrophobicity to a hydrophilic environment, and this change is accompanied by an increase in unsaturated carbon-oxygen bonds. Hence, oxidation of the C-C bond to a C-O is the first step to the enzyme-directed depolymerization process (Kim et al. 2020). Depolymerized products obtained from sunlight-treated oxo-biodegradable bags were taken up as metabolic substrates reinforcing the importance of the prooxidant state of the original polymer (Da Luz et al. 2014).

The attachment and growth of microbial colonies leading to the formation of biofilms also has an important place in plastic degradation. Surface hydrophobicity in plastics tend to discourage the colonization of bioentities to microparticles by PVA (Hüsler et al. 2018) and also by resisting non-specific protein adsorption via surface water (Shengfu Chen et al. 2006) which forms both a formidable physical and enthalpic barrier to invading microbes (Shenfu Chen et al. 2010). It has been reported, however, that certain synthetics support growth of bacterial colonies (Sivan et al. 2006). The lack of adhesion is overcome through the secretion of biosurfactants that take the form of lipopeptides and glycolipids from microbes that enable swarm mobility at the surface correlated to surfactant release (Be'er and Harshey 2011). The notion that plastics are not fully eliminated from the environment but persist as microplastics of the size order $\sim 100\text{ }\mu\text{m}$ in effluent waters (Wolff et al. 2019) adds another layer to the plastic problem. In an effort to ascertain how biofilm communities grow and effect polymer surfaces, microplastics derived

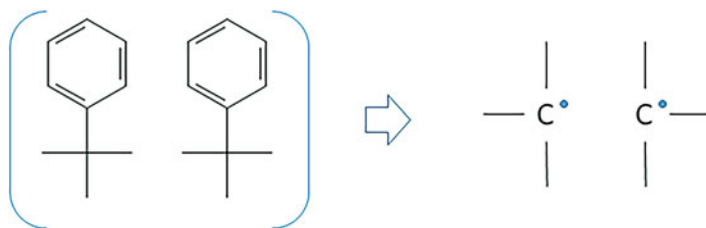


Fig. 9 Bond scission of polystyrene units forming carbon-free radicals as precursors to small metabolic products

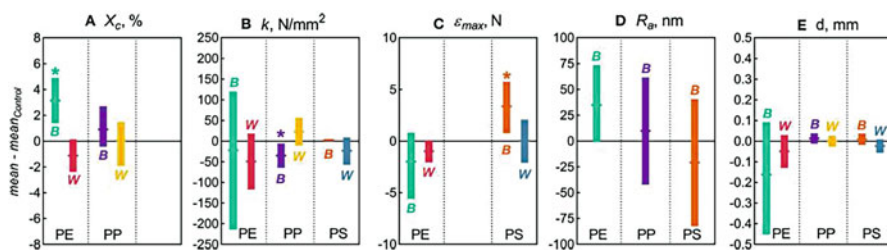


Fig. 10 Effect on polymer surface chemistry of PE (Polyethylene), PP (Polypropylene), and PS (polystyrene) measured by changes in (a) crystallinity, (b) stiffness, (c) compression, (d) roughness, and (e) diameter. The asterisks denote major differences in sample treatment relative to the control. (Reprinted from (McGivney et al. 2020). This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0))

from polyethylene [PE], polypropylene [PP], and polystyrene [PS] were incubated with sea water bacterioplanktons. Figure 9 shows that crystallinity, stiffness, and compressibility are affected significantly relative to control samples in water in the absence of microbes. The increased crystallinity of [PE] was notable in comparison to the control sample, and [PP] experienced the most significant loss in stiffness while the other polymers were similar to the control (McGivney et al. 2020). The loss in material property from the structural changes observed in this study and the irreversibility associated with this loss primarily from the breakdown of structure and mechanical properties suggest that degradation of smaller particles through the formation of biofilms is possible resulting in the variable accumulation of different bacterial colonies (Fig. 9). Specific effects are assigned to changes in surface chemistry and provide evidence for the attachment and degradability of polymers with strong dependency on the polymer structure. Hence, biofilm formation is polymer specific (Figs. 10 and 11).

These studies suggest that polymer biodegradation hinges on a number of factors, a single decisive factor rests on the structural and group composition, and the structural compatibility of the polymer-microbe is highly dependent on the nutritional source and energy requirements of microorganisms. Hence, the general limitation of polymer degradability might also be regulated by nutritional and energy requirements and the process may relate to a specific source type of plastic. This

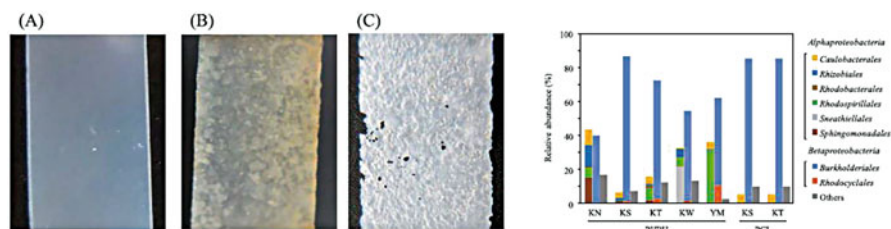


Fig. 11 The genesis of biofilm formation and accumulation on Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBH): (a) Untreated polymer; (b) biofilm after 2-week incubation; and (c) washed polymer exposing degraded regions. The RHS graph summarizes the relative accumulation of bacterial strains on the PHBH polymer. (Reprinted from (Morohoshi et al. 2018). This work is licensed under a Creative Commons Attribution International (CC BY))

indicates that synthetic polymers are not optimal substrates for secreted enzymes but rather are optimized for biopolymers that undergo rapid biodegradation such as chitin, cellulose, corn, starch alginate, etc. This requires the implementation of strategies and technologies to accelerate polymers that are not biologically designed for depolymerization but synthetically resistant in their makeup. One of the biggest challenges faced in the current era is the mass availability of alternative polymers that can easily replace plastics with sustainable properties that rival the mechanical characteristics and durability of polymerizable “hardcore” plastics. While polymer blending can deliver eco-friendly replaceable plastics, much effort is being directed to fabricate favorable polymers blends formulated with new elements for easy waste disposal with the same utilizable quality of existing plastics. Polymer blending is perhaps the only sustainable alternative to reduce and minimize environmental toxicity while partnering with the mechanics of natural living ecosystems to recycle plastic waste.

Polymer Blends Composed of Synthetics Polymers

Mainstream materials used for “everyday” packaging applications still remain essentially nonbiodegradable, and their replacement or their compatibility through modification is necessary to deliver environmentally safe and sustainable degradable products. Polymerizable monomers that can be environmentally degraded such as biodegradable polymers are extremely attractive to overcome these challenges. While synthetic polymers possess very strong bonding associations across the polymer backbone in traditional material such as polystyrene, biodegradable polymers are generally characterized by weaker bonds that allow them to satisfy their natural role as functionally degradable biomaterials. The replacement of synthetics with existing biodegradable materials is often hindered by inadequate mechanical properties and cost limiting their long-term durability. In response to the plastic crises, interest has intensified in recent years to chemically rationalize polymer with phase properties that favor their breakdown without damaging the marine and soiled

environments. The current approach is looking to overcome issues related to thermodynamic immiscibility which is often subject to thermodynamic aging in the face of nonstable interfaces that change physically with time (Vanhee et al. 2000).

Natural polymers fall into a number of functional group categories identified by their repeating chemical unit which are normally not programmable interconvertible with each other despite the adsorptive ability to interface with other chemicals, to show temperature and mechanical responsive behavior, and at the same time to resist change and retain their original structures which are strongly aligned to their chemical and physical properties. However, conditions are applied outside the stability range of the polymer by creating weak chemical links to liberate stable and reactive high energy radicals for creating new bonds in inert structures.

To address the process-cost factor relationship for achieving material sustainability for alternative polymers applicable in a broad commercial context, polymer blending finds considerable utility that makes use of a number strategies to enhance mechanical properties in particular (Paul 1992). Polymer composite technology is seeking to use base materials that are chemically different in tier properties and hold the potential to modulate both physical and chemical properties when strategically combined as a new biodegradable polymer blend. Man-made polymers that are not natural targets for biodegradation but can be chemically engineered to use biodegradable properties through compatible blending of functional are coming of age as the commercial is forced to move away from synthetics. While biodegradable polymers consist of many of the characteristics synthetic chemists aspire to incorporate into synthetic materials, the cost of making available natural biodegradable polymers is being challenged by tailoring chemistry itself to make synthetics susceptible to degradation via chemical routes using stimuli-responsive cleavable linkages – strategies that are becoming important for drug delivery (Zhang et al. 2012), cosmetics (Adli et al. 2020), and biomedical applications (Ju et al. 2009). Biobased polymers are poised to become the new bioplastics to replace petroleum of fossil-fuel plastics which have limited recyclability and utility after successive rounds of waste processing while decreasing the plastic grade quality. Selecting alternatives to “throw-away” plastics inevitably means choosing easily degradable components with limitless recyclability. To control the surge of petroleum-based plastics and their environmental impact, there has been a conscious shift to produce green plastics to replace their shift in product and food packaging and other sale products including toys and sports equipment. Such strategies open exceptional opportunities for overcoming these limitations with appropriate approaches to overcome barriers to their immiscibility, chemical compatibility, as it applies to performance-related behavior.

From an environmental point of view for the management of plastic degradation in soil, Varyan et al. (Varyan et al. 2021) proposed to use low-density polyethylene (LDPE) with natural rubber. With particular emphasis on low-strength synthetics, this approach makes use of low-strength polyethylene. The selection of the additive fits well with the rationale of weak bonds in LDPE in that the elasticity of the rubber additive reduces the elongation of break of the composite accompanied by increased water adsorption and caters for an efficient route for the biodegradability of PE in the

soil environment. However, soil degradation of natural rubber composites by microbes was reported to be dependent on filler content, filler particle size, and chemical treatment. Figure 12 shows the different types of enzymes that degrade natural rubber. Particularly, lignocellulosic fillers at the microscale played a prominent role in degradation, but chemical treatment significantly delayed microbial degradation of composites. Microvoids (revealed by SEM) indicated that tensile strength and hardness were sharply reduced facilitated through the formation of microvoids which also resulted in weight loss determined over a period of days (Sareena et al. 2014). Figure 13 shows an image of microvoids by scanning electron microscopy (Muniandy et al. 2016).

Natural polymers are prime targets for replacing fossil-based plastics for mainstream applications. Some of the more common pursuits are poly (lactic acid) (PLA), among others, pertinent to industry including polybutylene succinate (PBS), polybutylene succinate adipate (PBSA), polyhydroxy alkanates (PHAs), and polybutylene adipate terephthalate (PBAT), silk wool, Nylon-2-nylon-6, Poly β -hydroxybutyrate-co- β -hydroxy valerate (PHBV), cellulose, cyclodextrin, chitin, alginate, polysaccharides, starch, and rubber. The most recent consensus is that materials like PLA are feasible as replacements for synthetics for artificial bioplastics balanced by a high biocontent exceeding 80% (van den Oever and Molenveld 2017). The performance profile of a substituted biopolymer benchmarked against PS is shown in Fig. 14. The modeling of additives or modifiers in the substituted polymer signifies retainment of a high biocontent between 72% and 85%. This might be indicative of the unchanged biodegradation characteristics between mineralized and nonmineralized polymers, namely, PBS, PHBH, PBSA, and PBAT but marked an increase in the moduli state of the polymer (Ángeles-López et al. 2016).

There has been considerable interest for formulating novel biodegradable materials with the capability of enhancing mechanical properties of biodegradable blends which currently are limited by their fragile mechanical and thermal properties. In this respect, cellulose and chitosan (Nasalapure et al. 2020), their derivatives at the micro/nanoscale, exhibit water properties which may be coupled to their mechanical resistance. Polymer blends with short-life properties are of particular interest for tuning their biodegradability as an intrinsic property of the blend. However, some of the most efficient biodegradability rates are associated with biodegradable polymer blends (PCL-Starch) compared to the individual components (PCL and Starch) (Cho et al. 2011), and the inclusion of synthetics tends to drastically slow down depolymerization. Another ongoing concern with acidic biopolymers is the highly acidic nature of biodegradable products into the environment. While the chemistry of biosynthetic degradable polymers can potentially curb the release of acidity products through strategic polymer design, a congruent structure mimicking the blend lactic acid and glycolic was synthesized to lower the acidity of released products relative to both the natural polymer acids. The requirement for neutralization of biodegradable products of polyphosphazene/poly(alpha-hydroxyester) blend were reported to be significantly lower than those associated with the natural polymer acids (Ambrosio et al. 2002).

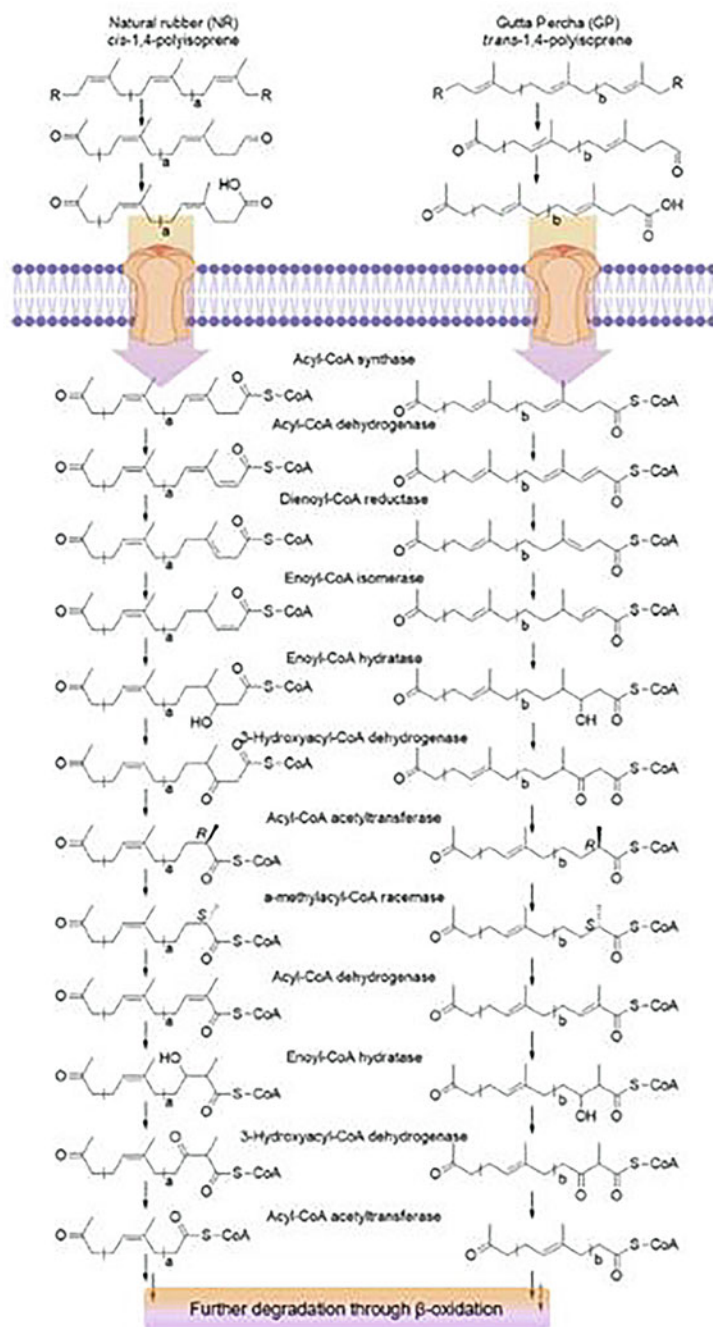


Fig. 12 Enzymes involved in microbial degradation of natural rubber. (Reproduced with permission from (Soares and Steinbüchel 2022))

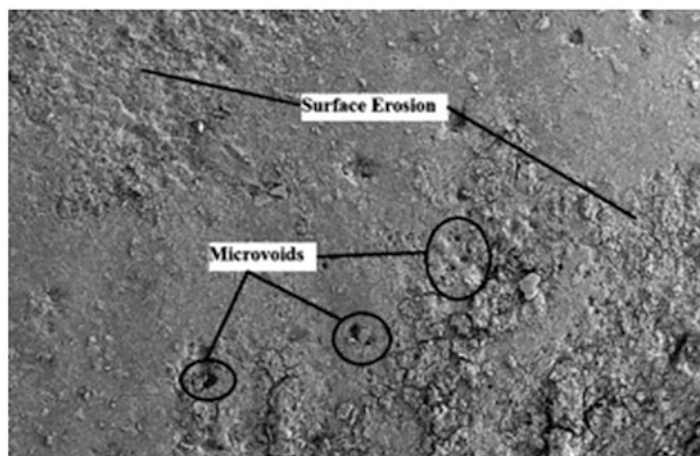


Fig. 13 (a) Scanning electron microscope image of natural rubber biocomposites. (Reproduced with permission from (Muniandy et al. 2016))

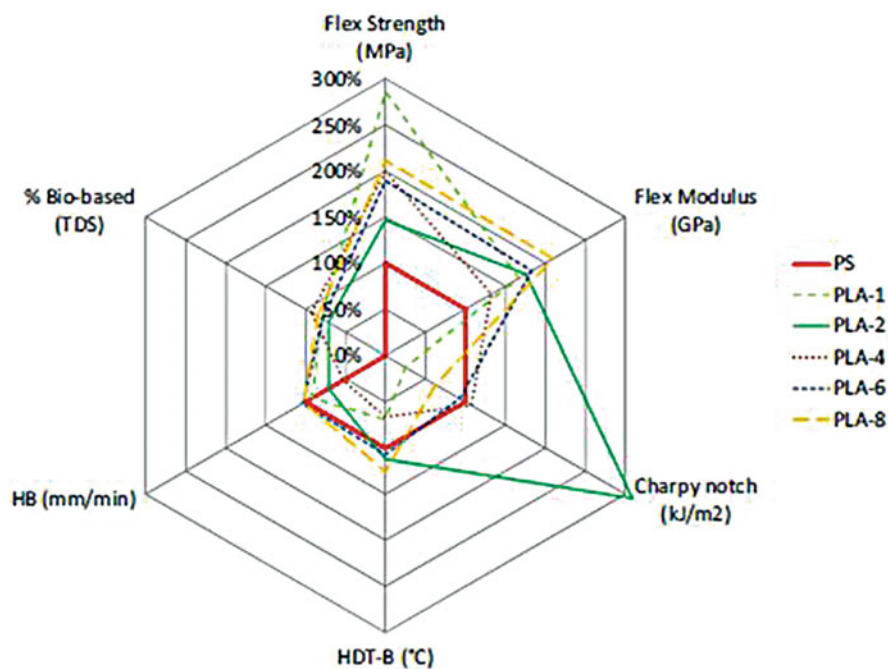


Fig. 14 Comparison of biobased content mechanical, thermal, and flammability of PLA performance against a synthetic reference PS. (Reproduced with permission from (van den Oever and Molenveld 2017))

Ideally, aligning the plastic properties of the polymer blend to suit its degradability under both biotic and abiotic conditions might be envisaged to broaden its applicability. This serves as an important goal to minimize the persistence of the synthetic component of the blend in diverse aquatic and soil environments that have variable effects on its degradability. For example, natural polymers may degrade differently under the same physical conditions (e.g., temperature) with varying degradation rates and accumulate differently in the environment. Biobased polymers are poised to become the new bioplastics to replace petroleum of fossil-fuel plastics which have limited recyclability and utility after successive rounds of waste processing while decreasing the plastic grade quality. Selecting alternatives to “throw-away” plastics inevitably means choosing easily degradable components with limitless recyclability. To control the surge of petroleum-based plastics and their environmental impact, there has been a conscious shift to produce green plastics to replace their shift in product and food packaging and other sale products including toys and sports equipment.

Controlled biodegradation and the release of nonharmful products from disposal plastics require a better understanding of the chemical and physical factors across all scales. Current and future objectives rest on understanding numerous aspects of polymer blends that relate to the composition type and methods used for blending, influence of blending ratios, and morphology and properties of composite; biodegrading rates and blends could be made smarter using analytical tools. A lack of understanding in the blending of biopolymers with synthetics such as that of starch with LPPE/TPS subjected to multiple cycles of extrusion processes does not often result in significant enhancement of mechanical properties (Peres et al. 2016) as desired. For example, AFM and ToF-SIMS have been used to conclude the distribution of surface energies in PLC and PVC blends and the location of Cl and O ions along the ridges and valleys of the blend (Chan and Weng 2016) (Fig. 15). Also, a leading limitation is in extending the utilization of polymer blends for the biodegradation of synthetics in distinguishing between miscibility, immiscibility, and complex mixed blends in between the extreme phases. In this context, Positron Lifetime Spectroscopy (PLS) and a hydrodynamic approach have been used to determine the level of interfacial adhesion (Ranganathaiah 2015) and to assess the potential for degradability. Such new techniques build upon knowledge relating to the compatibility of polymer blends using more traditional methods (Venkatramanan and Arumugam 2010). The method has been useful in establishing the degree of friction, repulsion, and attraction between biodegradable PCL and PVC components of the blend. For blends comprising a volatile solvent component, information is derived from processing conditions specific to blend ratio and evaporation rates. Computational approach has been insightful in relating such parameters to internal morphological patterns and to characterize emerging morphological types. The method therefore has considerable value in deciphering the mechanics of multistage processing, and often this information remains hidden and unavailable for process

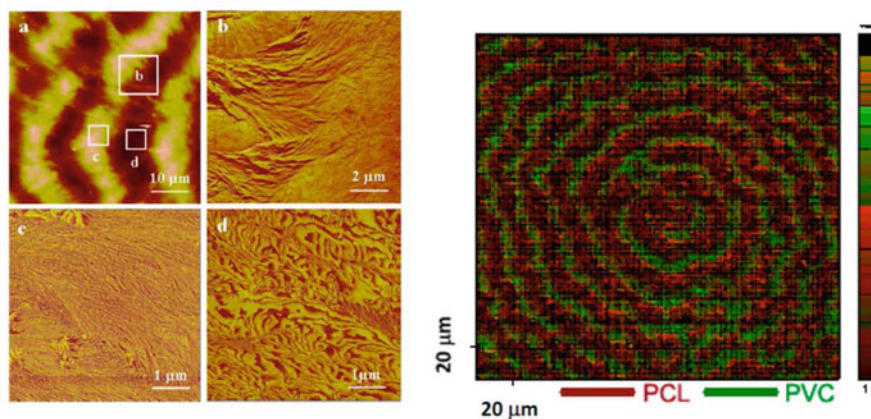
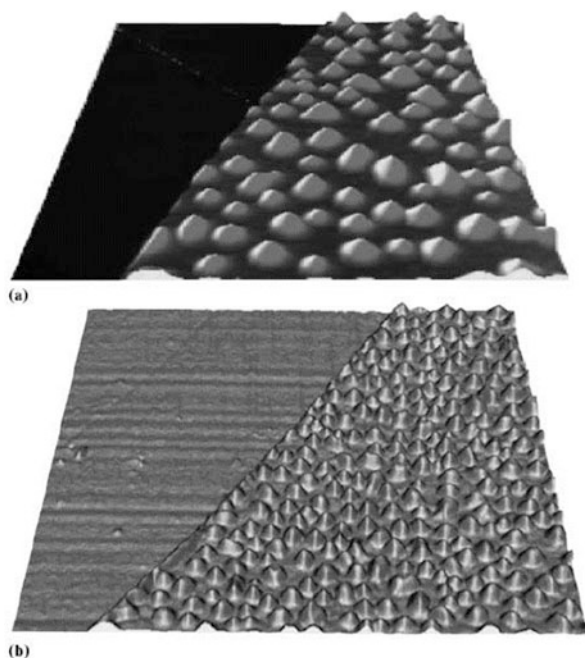


Fig. 15 The analytical application of atomic force microscopy (AFM) and time-of-flight secondary mass spectrometry (ToF-SIMS) in polymer assessing energy surface distribution in PCL and PVC blends. (Reprinted from (Chan and Weng 2016). This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0))

optimization (Pokuri and Ganapathysubramanian 2016). Additional factors driving the use of polymer blend technologies as future plastics not only reduce the carbon footprint of nondegradable plastics but also encourage the derivatization of CO and CO₂ while lowering energy requirements and temperatures, better by-product utilization of waste-degraded products. Efforts in this direction have seen new patented technologies emerging that take these into consideration by making use of polyolefins such as polyethylene (PE) and polypropylene (PP) as ingredients for blends and films with aliphatic polycarbonates (APCs) (Scott and Sanjeev 2013). For polymer blends that require a noninvasive route to their characterization, phase-sensitive acoustic microscopy can uniquely probe elastic subsurface material properties revealing a detailed contour with water as a coupling fluid (Ngwa et al. 2005), imaging difference between atomic force microscopy (AFM) and phase-sensitive acoustic microscopy (PSAM) (Fig. 16).

The advantage of using polymer blends composed of both a synthetic and a biosynthetic component is that the degradation of the biosynthetic counterpart which proceeds more readily necessarily assists in the fragmentation of the synthetic part allowing it to be more easily metabolized and thereby reducing the polymers bioresistivity. Polycarbonate (PC) is an example of one such biodegradable – a semicrystalline polymer that exhibits strong rigidity and mechanical toughness. However, the PC has long been recognized for its industrial importance especially as a modified polymer using polyester to generate compositions for molding with increased modulus and tensile strength, but transesterification between PC and polyester was considered undesirable (Bassett 1991). To improve its

Fig. 16 Comparison of the image topography PS / PMMA polymer blends using (a) AFM and (b) phase-sensitive acoustic microscopy. (Reprinted from (Ngwa et al. 2005). This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0))



physicochemical properties and cleavable properties under γ -radiation, PC-ethylene-vinyl acetate (EVA) blends, copolymer blends supplemented with abietic acid (Rosin) (20% Rosin/ 80% PC-EVA), were fabricated. Degradation of the copolymer under γ -radiation and the overall degradative properties of the copolymer are attributed to the photoreactive abietic acid moiety as demonstrated by the crosslinking activity of poly(glycidyl methacrylate) (Kim et al. 2005). The resulting photoactive copolymer, comprising a thermally stable low-modulus flexible EVA moiety, can potentially function as a biodegradable plastic modulated by the Rosin content (Nouh et al. 2016). An organic synthetic in the form of castor oil used as a plasticizer with starch derived from pumpkin was applied as a multicomponent composition to chitosan. Enhancement in the tensile strength of the composite was correlated to an increase in chitosan with an optimum starch-to-chitosan ratio of 50: 50, respectively Here, the ease of microbial biodegradability of the composite in the culture medium was correlated to be a function of the glucosidic bond content of the amylose and amylopectin units of starch and was counterbalanced by the Young's modulus which was strongly influenced by the presence of chitosan (Hasan, Rahmayani, and Munandar 2018). The role of starch degradability was supported by the bioplastic performance of a starch / chitosan composite reinforced by polypropylene as the synthetic component. The optimal ratio of starch/chitosan was established as 65/35 and displayed mechanical properties that were well-suited for material degradation under soil burial demonstrating a high percentage (>80%) after 28 days (Fig. 17).

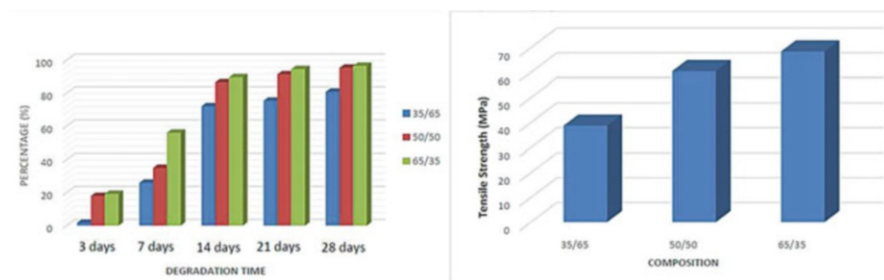


Fig. 17 (a) Biodegradation rate and (b) composition of polypropylene reinforced starch / chitosan composite. (Reprinted from (Jangong et al. 2019). This work is licensed under a Creative Commons Attribution 3.0 License (CC BY 3.0))

Potential for Smarter Polymer Blends for Controlled Depolymerization

A novel route to degradability of polymers reportedly supports the existence of mechanically gated degradable polymers. The concept relies on combining a stimulated trigger degradation which is further enhanced by a site-specific mechanical force applied on the polymer backbone. In this specific example, cyclobutane (CB) serves as the mechanopore which acts as a “mechanical gate” that modulates an acid-responsive ketal group integrated into the polymer backbone. It has been evidenced that the acid component alone does not elicit the optimum breakup of the polymer in the absence of an ultrasonication step delivering the mechanical trigger (Fig. 18) (Lin et al. 2020b).

The observation also raised a pertinent question whether mechano-triggered bond scission leading to polymer fragmentation was confined to only to single site degradation or whether multiple site depolymerization was also a phenomenon. To shed further light on this area, in a follow-up study, a [4.2.0] bicyclooctene (BCOE) was investigated for properties as a mechanophore. In an exquisite study, Lin et al. (2020a) performed mechanical degradation on the solvent-treated polymer by ultrasonication and reasoned the effect of supplying a mechanical-based trigger for the type of polymer chosen should (a) result in the reduction of the MW, (b) fragmentation will lead to their respective mechanophore products, and finally (c) result in a ring opening of cyclobutane core of BCOE. The increase in sonication expectedly caused an increase in the rate of events (a), (b), and (c) followed by a decrease in rate as result of polymer fragmentation. In fact, it was revealed that the liberation of smaller molecular weight oligomers from stress-induced fragmentation via ultrasonication was operational giving credibility to the existence of mechanophores. The mechanism that was originally thought to be restricted to single-site scission is in fact driven by collective forces over a timescale of 221 h that when delayed, polymer degradation occurs very slowly resulting in multiple scissions across the length of the polymer. Mechanical degradation via this

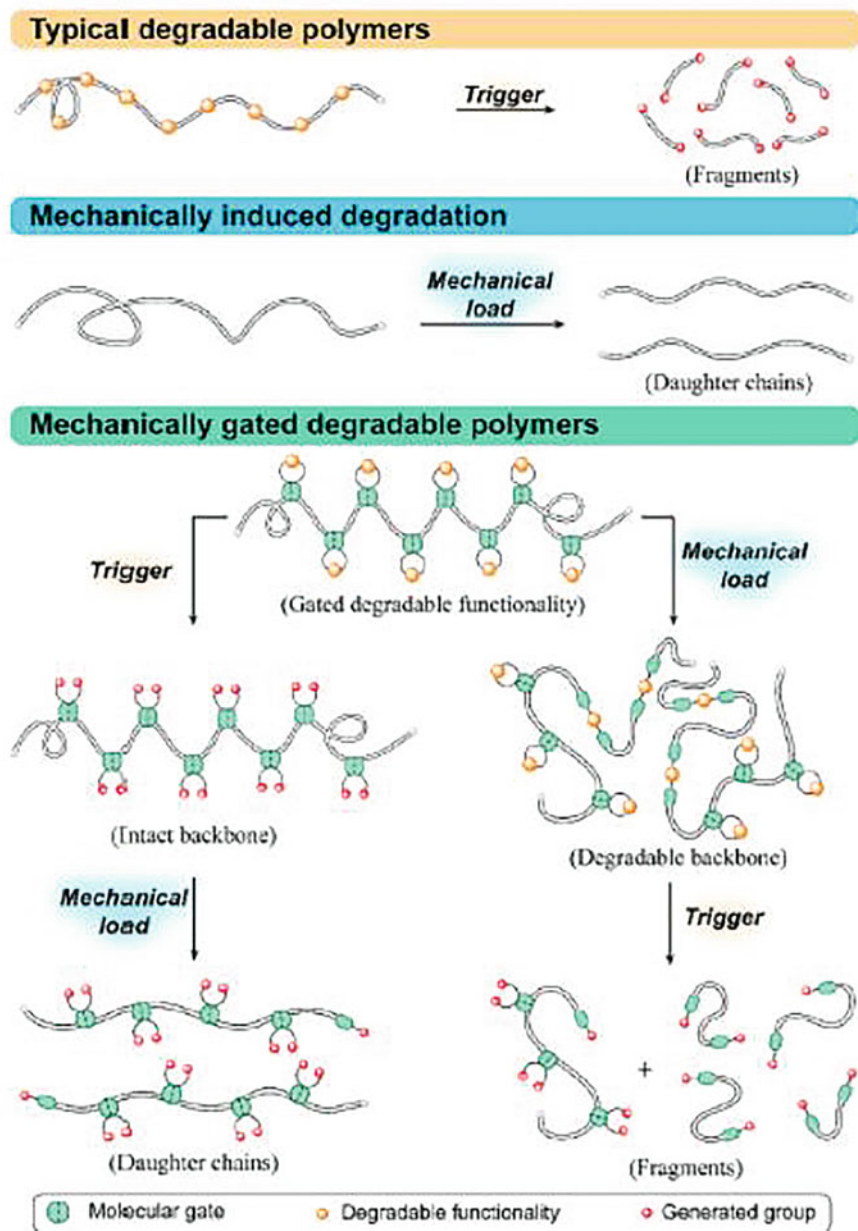


Fig. 18 Schematic mechanism describing polymer backbone fragmentation by a mechanically gated mechanism. (Reprinted from (Lin et al. 2020b). This work is licensed under a Creative Commons Attribution License (CC BY))

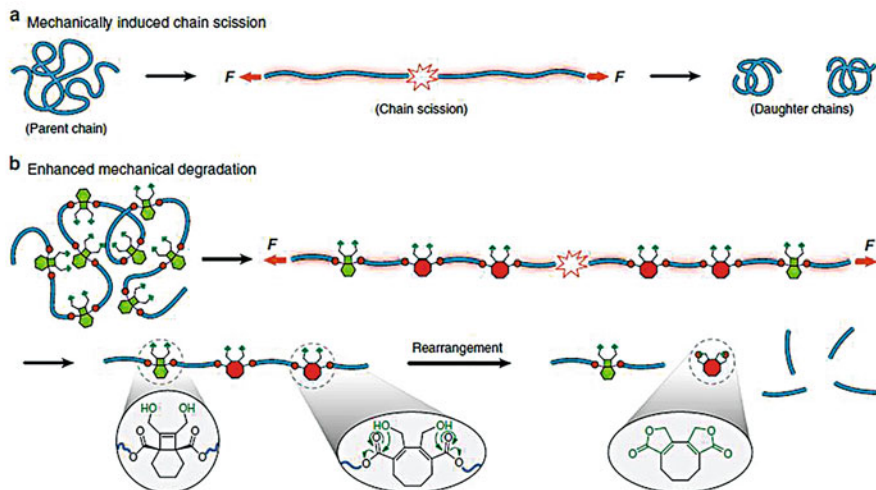


Fig. 19 A schematic description of comparing (a) mechanical induced chain scission and the (b) mechanistic enhancement of polymer fragmentation via a multisite bond scission process. (Reprinted from (Lin et al. 2020b)). This work is licensed under a Creative Commons Attribution License (CC BY)).

mechanism allows the degradation of polymers, and in this case multiple fragments were identified tenfold smaller than the original polymer. The mechanism is summarized in Fig. 19.

Concluding Comments

Depolluting the earth begins with drastically diminishing nondegradable plastic accumulation. The chemical merger of synthetic and biobased polymers materials in the form of biosynthetic blends to induce artificial and tuneable properties is just being understood. The quality and performance of the resulting polymer depends on selection of the blend, and the shift to biodegradable polymers to replace plastic-based materials derived from fossil derivatives is currently in development. There is tremendous interest in the application of polymer blends particularly toward the biodegradable plastics. The strategic blending of synthetic and biodegradable polymers continues to evolve but at a slower pace solely to their biodegradable counterparts. Material capability depends on deciphering complex factors to develop highly degradable plastics that also have synthetic value that provide the necessary mechanical support and rigidity that biodegradable plastics lack. To make progress, the evolution of analytical tool is also important to understand complex interfaces to optimize both the structural and functional performance of highly degradable blends. In the advancement of this technological pursuit to implement functionally

competent eco-friendly biodegradables, engineering the design of smarter polymer blends should be a prime objective while exploring common and cost-effective synthetic materials.

Cross-References

► Biopolymer Waste Management

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Biopolymer-Based Interpenetrating Polymer Networks

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T. R. Anju and J. Sindhu Rachel

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T. R. Anju (✉)

Department of Biotechnology, Newman College, Thodupuzha, Kerala, India
e-mail: anju.tr@newmancollege.ac.in

J. Sindhu Rachel

Department of Botany, Nirmala College, Muvattupuzha, India
e-mail: sindhu@nirmalacollege.ac.in

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Abstract

Interpenetrating networks (IPNs), the blend of cross-linked polymers, exhibits unique attributes as these networks can retain the properties of constituent polymers and can offer new features of the polymer blend. IPNs are usually fabricated by sequential or simultaneous method and can produce different types like full IPN, semi-IPN. Based on the constituents and pattern of cross-linking, each IPN shows its own characteristic properties. One of the emerging areas in IPN fabrication is the use of biopolymers like polysaccharides, proteins, or oils in the polymer blend. Among the various biopolymers, many polysaccharides like alginate, chitosan, starch, xanthan gum are extensively used for IPN fabrication. Proteins like fibrin, silk fibroin, and gelatin are also used in many interpenetrating networks. These biopolymer-based IPNs are mainly used in biomedical fields in sustained and targeted drug delivery, tissue engineering, and gene delivery due to its biocompatibility and biodegradability. Nowadays, cellulose-based IPNs are fabricated to develop cost-effective sustainable alternative to plastics in food packaging industry. The benefits of these biopolymer-based IPNs in various fields, especially biomedical field, can be explored in depth with more detailed investigation in this area of polymer chemistry.

Keywords

Polymer blends · IPN design · Polysaccharide IPN · Protein IPN · Drug delivery · Tissue engineering · Food packaging

Introduction

Polymers are extensively used in many industrial sectors and are considered to be the most widely used chemical product with a growing demand in global market. The versatile properties exhibited by polymers and its various assemblies contribute to its immense industrial applications including biomedical and therapeutic applications. Many polymers are extensively used in biomedical applications as excipients in formulations of capsule and tablet (Kumar and Kumar 2001), as enhancers of blood circulation time (Blume and Cevc 1990) and in targeted drug delivery system (Liechty et al. 2010). Naturally occurring biopolymers, produced by living organisms, have fascinated the scientific world with its ability to design novel polymer-based materials due to its biodegradable, biocompatible, and nontoxic properties. Amidst all these, continuous efforts are going on to improve polymer qualities like biological performance and mechanical strength. This led to the design of new class of polymers by blending various polymer mixtures, either natural or synthetic polymers alone or in combinations, to get cross-linked polymers (Giusti et al. 1996).

Interpenetrating polymer networks (IPNs) are exclusive blend of polymeric substances in which a polymer network is cross-linked to another. IPNs are unique in its properties as it confers the key attributes of one polymer by maintaining the essential attributes of other polymer in the blend. Occasionally, totally newer properties, than any of the two polymers used for blending, are exhibited by the IPNs. Thus, developing interpenetrating polymer networks system using various polymers provides a perfect method to obtain materials with wide range of characteristics to improve existing properties and to overcome the demerits of individual polymer network. The better qualities exhibited by IPNs over the conventional individual polymers have contributed to its high demand for various applications. The superior qualities of IPNs like highly compatible, degradable, and stable nature within biological systems and nontoxic nature point to the intense demand for these materials in the production of new deliverance mechanisms for controlled drug release studies (Lohani et al. 2014). This chapter encompasses an overview about IPNs, its types, design, and characterization of IPNs, biopolymer-based IPNs, and its biomedical and other applications.

Interpenetrating Polymer Networks (IPNs)

An IPN is defined as an arrangement of polymers in such a way that one (or more) polymer is produced in the presence of another to form a network with cross-links (Sperling and Hu 2003). Here, the polymer chains of one system intertwine the network formed by another polymer. The uniqueness of such system is the ability to retain individual properties by both polymers and sometimes synergism is also observed for properties like mechanical strength or toughness (Ignat and Stanciu 2003). An IPN is different from a usual blend of polymer with its capability to swell without suppressing flow or getting dissolved in the solvents (Kudela 1987).

IPNs are different from the complexes or grafts of polymers/copolymers, which have chemical bonds and/or partial cross-linking. Thus, IPNs are considered as “polymer alloys” where blends of polymers are made chemically compatible to achieve the desired phase morphology (Work et al. 2004). IPNs are different from other multiple systems due to its continuous cross-linked structure formed by two polymers of intimate contact without any chemical bonding, which can produce a novel material of improved qualities depending on composition and degree of cross-linking (Fig. 1). Hence, we can consider IPNs as entangled polymer networks holding to each other by stable peripheral interactions. The chemical interactions between polymers make the IPN a stable entanglement that needs breakage of one or more networks to separate them.

Generally, polymers are difficult to mix with each other, resulting in phase separation of blended polymers, and hence, miscibility of the interpenetrating polymers determines the success in fabricating IPNs (Olabisi et al. 1979). Thus, cross-linking plays a pivotal role in enforcing the miscibility of two polymer materials, which are normally immiscible, and many combinations of polymers can be explored by this cross-linking to identify IPNs with unique properties and applications. Fabrication of IPNs using biopolymers from various sources is an area of immense potential due to its sustainability and biocompatibility.

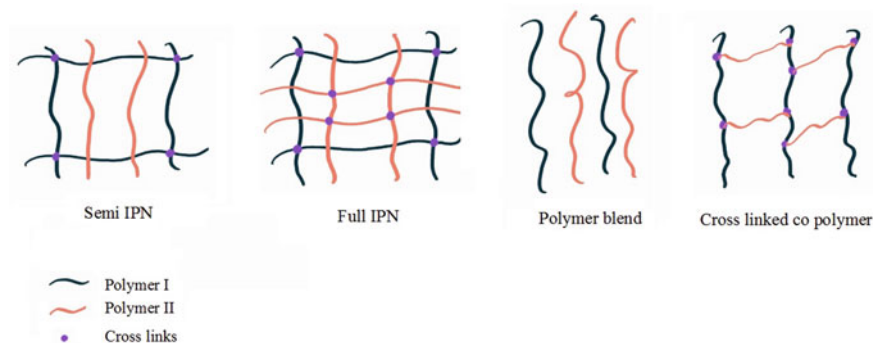


Fig. 1 Schematic representation of various polymer networks like interpenetrating networks, blend and cross-linked copolymer

The idea of interpenetrating polymer network was first introduced by Aylsworth as early as 1914 by fabricating a mixture of natural rubber, sulfur, and partly reacted phenol-formaldehyde resins (Aylsworth 1914). It was only in 1960s that these cross-linked networks were named as interpenetrating polymer network by Miller based on his scientific study about polystyrene networks (Millar 1960). Later on, IPNs have been an area of great interest in the field of material design with new combinations emerging at a fast pace for various applications in different sectors. Now, the field of polymer science is witnessing an ever increasing quest towards devising new IPNs with better properties. IPNs are ideally permanent entangled networks of polymers held together by topological interactions (Sperling et al. 1972). The inter-network entanglements are permanent by virtue of the fact that the two networks are themselves chemically cross-linked and thus cannot be “pulled apart” from each other without fracturing one or both of the networks.

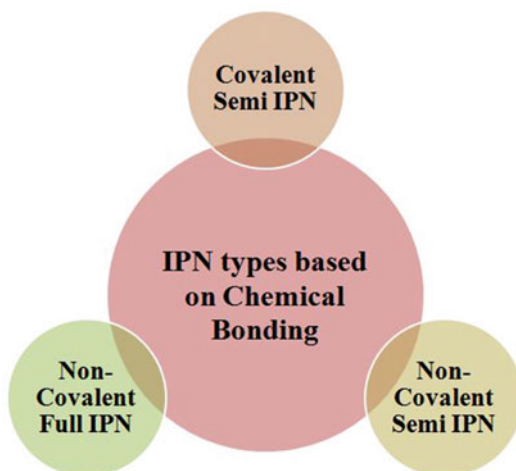
Major Features of IPN

Ideally, when polymers are blended together to form IPN, creep and flow movements are suppressed. When mixed with a solvent, IPNs exhibit swelling and do not dissolve. IPNs mainly form insoluble network with high tensile strength and adhesive property. Most of the IPNs are heterogeneous systems with two phases: rubbery and glassy to get perfect synergism in the blend. The number and nature of cross-links determine the differences between various IPNs. Due to its unique properties, IPNs are suitable for controlled release of compounds like drugs.

Classification of IPNs

Intensive research in polymer technology resulted in the development of different forms of IPN for various applications. A lucid classification of IPNs into different categories is difficult, but it can be broadly classified based on the chemical bonding or pattern of arrangement due to different synthesis methods.

Fig. 2 Schematic representation of types of interpenetrating networks based on chemical bonding



Based on Chemical Bonding

IPNs can be formed by irretrievable chemical links formed by covalent or non-covalent chemical bonds.

The primary goal of these chemical bonding is to ensure proper entrapment of the absorbed water and carrier molecules and its controlled release on stimulation by simple diffusion. The different IPNs based on chemical bonding are described below (Fig. 2).

Covalent Semi-IPN

In this, covalent bonds link two different polymers resulting in one cross-linked polymer network.

Noncovalent Semi-IPN

In this, only one polymer system in the polymer network (comprising of two or more polymer systems) forms cross-links.

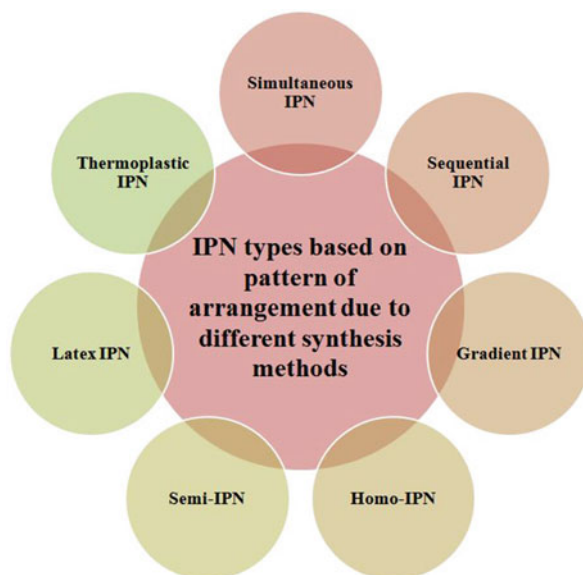
Noncovalent Full IPN

Here, all the polymers (usually two polymers) used for IPN fabrication will form cross-links in an independent manner to form a full IPN.

Based on the Pattern of Arrangement

There are different methods for synthesis of IPNs and this contributes to different patterns of arrangements (Fig. 3). Accordingly IPNs can be classified as follows:

Fig. 3 Schematic representation of types of interpenetrating networks based on pattern of arrangement



Simultaneous IPN

In this process, all the components for IPN synthesis are mixed simultaneously. In a single step, all prerequisite materials like the polymers, its cross-linkers, and activators are added under optimal conditions for effective fabrication of IPN mediated by noninterfering concurrent polymerization reaction (Tang et al. 2009).

Sequential IPN

Here, the production of IPN occurs in a sequential manner, that is, the monomers of one polymer polymerize to form the first polymer network and this is followed by the next polymer. This can be achieved by simple blending of materials. The first polymer system will swell in the presence of the monomers of second polymer, and the activators and cross-linkers will in turn form the entangled networks (Patri et al. 2007).

Gradient IPN

In this type, one monomer network undergoes swelling inside second network and the swelling stops before attaining equilibrium. Polymerization will happen at this stage resulting in an IPN in a gradient pattern (Karabanova et al. 2005).

Homo-IPN

This is the model IPN where the monomers of two polymers formed structurally similar networks (Dragan 2014).

Semi-IPN

Semi-IPN is formed by cross-linking one or more polymers (sequentially or simultaneously), exhibits a linear structure (Bashir et al. 2017), and usually involves the polymerization of a monomer in the presence of a polymer. Hence, one of the components has a linear structure instead of a network structure. Sometimes only IPNs designed by sequential process are called semi-IPNs and those made by a simultaneous process are called pseudo-IPNs.

Latex IPN

Since IPNs are thermosetting polymers, it is very difficult to mold it after formation. This issue can be addressed by using latex IPNs where each micro-IPN is a particle prepared in the form of latex. In this method, the first cross-linking monomer of the original seed of latex along with cross-linking agent and initiator will get bound by the second monomers by polymerization technique to get IPN networks of the single latex particle. It is also termed as an interpenetrating elastomeric network (Sperling et al. 1972). Latex IPNs are formed from a mixture of two lattices, frequently exhibiting a “core” and “shell” structure. In a sequential method, if the monomers corresponding to the second polymer react near the surface of the first polymer, latex IPN with shell/core morphology will be obtained (Lipatov and Alekseeva 2007).

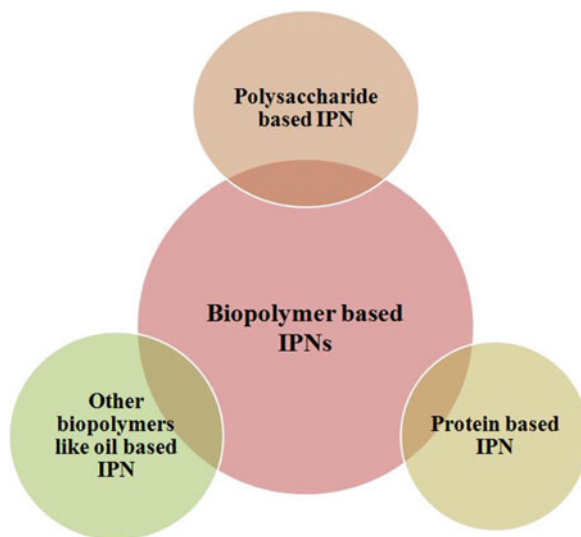
Thermoplastic IPN

Thermoplastic IPNs can be molded, extruded, and recycled. In this type of IPN, thermoplastic elastomers are used as physical cross-linkers instead of chemical cross-linkers. The thermoplastic IPN involves two physically cross-linked polymers which arise from an ionic group, glassy domains, and crystallinity. In this type of IPN, the materials flow at elevated temperature, behave like a conventional thermoset IPN where one element is a block copolymer and the other one is the semi-crystalline or a glassy polymer (Ohlsson et al. 1996). Depending on the continuity and proportion of phases, this kind of IPNs can exhibit a wide range of properties, from reinforced rubber to high impact plastics.

Biopolymer-Based Interpenetrating Networks

The first IPN resin developed by Aylsworth in 1914 was produced by heating a mixture of rubber, sulfur, phenol, and formaldehyde to get simultaneous interpenetrating polymer network. Later on, many researchers fabricated different IPNs using bio-polymers, a mix of biopolymers, synthetic polymers, or various mix of polymers to get final products with desirable properties. Semi-interpenetrating Polymer Networks and Interpenetrating Polymer Networks of multicomponent hydrogels are emerging as preferred biomaterials for drug deliverance and tissue engineering. The physico-chemical properties of the IPNs obtained by chemical or physical cross-linking show many desirable characteristics different from those of the macromolecular constituents. Fabrication of various types of IPNs opened a lot

Fig. 4 Schematic representation of biopolymer-based interpenetrating networks



of possibilities in the field of biomaterial research. Many polymers and its precursors with hydrophilic (water loving) nature are used to make hydrogels, most of which belong to natural polymers like polysaccharides and proteins and its derivatives and synthetic polymers containing hydrophilic functional groups. Due to the biocompatibility and biodegradable properties of biopolymers, a lot of attention was given in the design of biopolymer-based IPN especially for applications in therapeutics and tissue engineering. Different biopolymers like polysaccharides, proteins, oils are widely explored for the synthesis of IPNs (Fig. 4). Among various biopolymers, the fabrication and applications of polysaccharides and protein-based IPNs are studied in more detail owing to its ease of availability and properties.

Polysaccharide-Based IPNs

Among the different biopolymers that are used for the fabrication of IPNs, polysaccharides like chitosan, alginate, and starch attracted researchers a lot due to its availability from renewable sources in a large scale and its flexibility for chemical modifications. In the recent years, many polysaccharides were reported to be the material of choice to design IPNs for biomedical applications. The following are the most studied polysaccharide-based IPNs.

Alginate-Based IPNs

Many naturally derived polymers from polysaccharides have been evaluated and extensively used for preparing IPNs for various applications especially in biomedical

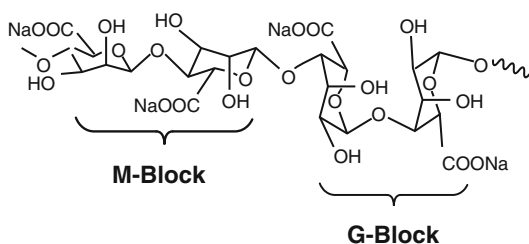
fields. Among these, alginate, a group of naturally occurring anionic polysaccharides, is reported to be highly useful to devise IPNs for drug delivery. The ability to form porous networks of high stability and biocompatibility makes sodium alginate-based IPN hydrogels a suitable choice for sustained drug release to target sites without altering the pharmacological effects of the drug.

Chemically, sodium alginate is a linear copolymer of 1,4 β -D-mannuronic acid (M) and α -L-guluronic acid (G) residues (Stanisci et al. 2020) (Fig. 5). The favorable properties like compatibility to biological system and drugs and nontoxicity inspired scientists to investigate more on the development of sodium alginate-based IPN hydrogels.

IPN hydrogels with augmented properties, especially biomedical properties, can be designed by combining sodium alginate with other polymers. Some examples of compounds with high drug encapsulation rate are the combination of sodium alginate with gelatin (Bhutani et al. 2016), or hydroxypropyl methyl cellulose (Hu et al. 2018) and chitosan (cationic polysaccharide polymer) coated beads of sodium alginate (Ramesh et al. 2017). The cross-linked network of sodium alginate with ovalbumin is also reported to be successful in designing alginate-based IPNs (Fang et al. 2007).

In addition to the abovementioned natural polymers, IPN hydrogels of alginate was prepared using combination with different synthetic polymers too (Samanta and Ray 2014). A sequential method of synthesis was used by Kim et al. to fabricate an IPN hydrogel of sodium alginate and poly(diallyldimethylammonium chloride) (PDADMAC), which exhibited response to change in physical parameters like temperature and pH (Kim et al., 2004). Many desirable properties like high porosity (Yin et al. 2007), controlled drug release (Wang et al. 2009), high electrical sensitivity (Kim et al. 2004), and responsive to multiple parameters (Dumitriu et al. 2011) have been achieved by designing sodium alginate-based IPN hydrogels with synthetic polymers containing carboxylic groups. Sodium alginate-g-poly (vinyl caprolactam) IPN beads were fabricated by Swamy et al. as an anticancer drug by exploiting its thermoresponsive nature, where a temperature of 25 °C triggers drug release rather than the usual body temperature of 37 °C. Chen et al. demonstrated the protein drug delivery using an IPN hydrogel of alginate with water-soluble chitosan derivative (N, O-carboxy methyl chitosan) in a pH sensitive manner (Chen et al. 2004). Thus, IPN composite hydrogels of sodium alginate and other natural or synthetic polymers were widely studied and applied in biomedical fields.

Fig. 5 Structural unit of sodium alginate



Chitin-Based IPNs

Chitin is the natural polysaccharide composed of β (1 \rightarrow 4)-linked 2-acetamido-2-deoxy- β -D-glucopyranose units (N-acetylglucosamine) (Dutta et al. 2004). The principle difference of chitin from the most abundant polysaccharide, cellulose, is the presence of acetamide group ($-\text{NHCOCH}_3$) instead of a hydroxyl group at the C2 position. Chitin is considered as a homopolymer even though some of the glucopyranose residues show deacetylation and exists as 2-amino-2-deoxy- β -D-glucopyranose. The degree of acetylation in chitin is generally 0.90, signifying the presence of amino groups (around 5–15%) (Franca et al. 2008). When the degree of acetylation in chitin is very low, it leads to the protonation of amine groups. This produces chitosan, a soluble polysaccharide compound which is slightly different from chitin (Hajji et al. 2014) (Fig. 6).

Both chitin and chitosan are natural polymers with excellent properties like biodegradability, biocompatibility, nontoxicity, and adsorption. One of the successful approaches to improve the quality of chitin-based materials is to combine it with other polymers or particles to get IPN. When the combination is carefully optimized, these interpenetrated polymer networks can give many desirable properties of both polymers in the final material. The chitin and lignin IPN film developed by Duan et al. showed a high adsorption capacity for ferric and cupric compounds from aqueous media (Duan et al. 2018). They developed the films by dissolving lignin and chitin as such (without any modification) in an ionic solution of 1-butyl-3-methylimidazolium and γ -valerolactone. In this film, the presence of chitin contributed to the flexibility and high metal adsorption capacity even at low concentration. The film also exhibited properties like stability in aqueous solution, ease to use, facile desorption, and reusability, which makes it a suitable IPN for water purification processes.

Another interesting blending of polymers is chitin with cellulose due to the insoluble nature of both these natural polymers (Liang et al. 2007; Zhou et al. 2004). Zhou et al. designed a fixed bed column using this blend which can effectively remove heavy metal ions and can regenerate the metal ions from the adsorption column using aqueous solution of 0.1 mol/L HCl. Liang et al. fabricated cellulose/chitin blend membranes, which is biodegradable and highly porous by

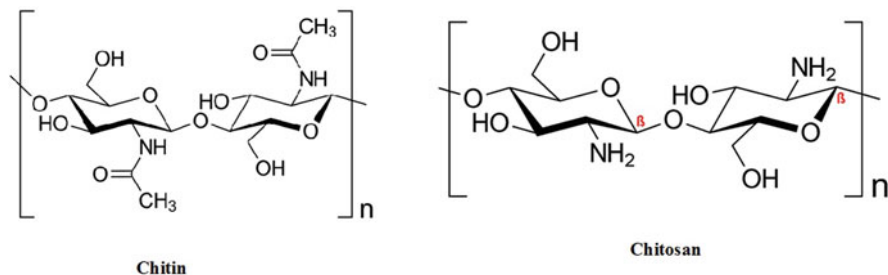


Fig. 6 Structural units of chitin and chitosan

blending both polymers in presence of aqueous solutions of 9.5 wt.% NaOH/4.5 wt.% thiourea followed by coagulation with 5.0 wt.% $(\text{NH}_4)_2\text{SO}_4$ (Liang et al. 2007). Researchers have observed that addition of chitin in blend membranes can produce materials of varied permeability due to the modifications in morphology and crystalline nature.

Chitosan-Based IPNs

Chitosan-based IPNs have been extensively studied for its biomedical applications as a suitable drug delivery system. Chitosan exhibits favorable features like the ability to adsorb chemicals like dyes, metal ions, and proteins (biosorbent) due to the presence of amino and hydroxyl functional groups (Crini and Badot 2008) and its IPN hydrogels can act as excellent drug delivery systems.

Chitosan was blended with cellulose (Cai and Kim 2009), its derivatives (Angadi et al. 2010), or acrylamide grafted -dextran (AAM-g-dextran) (Rokhade et al. 2007) using glutaraldehyde as the cross-linker for IPN fabrication. Semi-IPN hydrogels of chitosan and AAM-g-hydroxyethyl cellulose (AAM-g-HEC) showed increased loading capability/encapsulation efficiency with diclofenac sodium (Al-Kahtani Ahmed et al. 2009). In vitro studies on this IPN showed that the drug release is dependent on the ionic strength, the amount of AAM-g-HEC used, degree of cross-linking, and drug loading. Another study targeted the preparation of IPN hydrogels of polyethylene glycol (PEG) grafted on carboxymethyl chitosan and alginate which can discharge drug at pH 7.4, suggesting the feasibility for intestine targeted drug delivery (Yang et al. 2013). In many studies, glutaraldehyde is used as cross-linker for chitosan IPN synthesis due to the fast development of Schiff base between the amino groups of chitosan and aldehyde groups of glutaraldehyde. The high toxicity of glutaraldehyde prompted researchers to find better alternatives like the natural cross-linking agent, genipin in the preparation of chitosan-based hydrogels (Cui et al. 2014).

Chitosan-based IPNs were made with other polymers and tested for its biomedical applications. IPN beads of chitosan-glycine-glutamic acid were prepared with glutaraldehyde as cross-linker for controlled release of compounds. The study observed that the swelling properties and drug release were dependent on acidic strength, degree of cross-linking, and constituent composition (Rani et al. 2011).

In another report by Reddy et al., emulsion-cross-linking was exploited to make IPN microparticles of Ghatti gum and chitosan which were used for intestinal deliverance of diclofenac sodium. This study reported a prolonged drug release up to 12 h (Reddy et al. 2013). Rusu et al. developed IPN hydrogels of poly (2-dimethylaminoethyl methacrylate) and maleoyl-chitosan (water soluble) with self-healing ability, high stability, and exceptional environmental responsiveness. The self-repair ability along with dual pH/thermo-responsiveness and biocompatibility of these hydrogels was suggested to have many bio-applications (Rusu et al. 2019).

Starch-Based IPNs

Starch, the second most abundant polysaccharide, is a water-soluble, bioactive, biodegradable, and biocompatible polymer. Starch granules are made up of amylose and amylopectin: 20–30% consists of linear chains of α -(1–4-linked-D-glucose) units called amylose and remaining is made of branched chains of α -(1–4-linked-D-glucose) units interlinked by α -(1–6-linked-D-glucose) linkages called amylopectin (Fig. 7).

Researchers have carried out many modifications in native starch to improve the hydrophilic nature (Reis et al. 2008) and are used in the fabrication of biodegradable hydrogels for therapeutic applications (Soleimani and Sadeghi 2012). The IPN hydrogels or semi-IPN of starch are used for several applications due to its improved mechanical properties and solute diffusion (Jin et al. 2013). Another group fabricated a semi-IPN hydrogel by cross-linking acrylamide and sodium methacrylate in the presence of starch and observed the swelling/diffusion properties (Keshavara Murthy et al. 2006) and recommended its use as moisture maintenance materials in agriculture fields. Many studies were carried out in various semi-IPN hydrogels composites with starch to study the swelling capability and pH-sensitivity. These studies revealed the efficient swelling capacity and excellent pH-sensitivity of some starch-based semi-IPN hydrogels (Li et al. 2008).

Recently, Wei et al. fabricated starch-cellulose IPN hydrogels by sequential Diels-Alder click reaction and photopolymerization in water. Starch was modified with N-maleoyl- β -alanine to get starch-based dienes, and reaction of cellulose and furfurylamide succinate gave cellulose-based dienophiles. These two along with polymerizable β -cyclodextrin, cross-linker, and acrylamide were dissolved in water to obtain a transparent solution, which was kept at 50 °C for 3 h for Diels-Alder reaction, and then, the photopolymerization was done to get the second network. The hydrogels prepared were checked for the efficiency for sustained release of model drug, 5-fluorouracil. The simple method of catalyst-free, photopolymerization reaction, which can be controlled, was found to be a favorable technique for starch-cellulose IPN hydrogels fabrication (Wei et al. 2022).

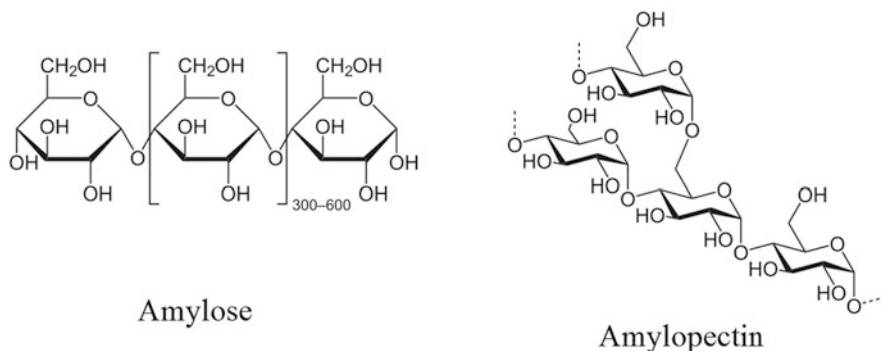


Fig. 7 Structural components of starch

Carrageenan-Based IPNs

Carrageenan (CG) is a polysaccharide biopolymer of high molecular weight obtained from seaweeds. Its monomers, galactose, and 3,6-anhydrogalactose (3,6 AG) are arranged in a linear fashion by alternate α -(1,3) and β -(1,4) glycosidic linkages (Bajpai and Pradeep 2013). The name of this polysaccharide is derived from carrageen moss (Scientific name: *Chondrus crispus*), a seaweed species. There are three types of carrageenan: kappa (κ), iota (ι), and lambda (λ) (Fig. 8). κ -carrageenan has the capability to form thermoreversible gel and is commonly used in food industry and pharmaceuticals (Van de Velde et al. 2002) due to its favorable properties like gelling, viscosity, and biocompatibility and biodegradability (Gupta et al. 2001).

The λ -CG can form viscous solutions which cannot form gels, whereas the κ -CG can form a fragile gel. The ι -CG can form elastic gels (Necas et al. 2008).

Carrageenan-alginate IPN hydrogel beads, which can release drugs by controlling pH of the media, were fabricated by Mohamadnia et al. to investigate the potential to deliver standard drug betamethasone acetate. The group studied the properties of the IPN using various analytical techniques and observed the most efficient drug loading capability at a pH of 4.8 and temperature of 55 °C (Mohamadnia et al. 2007). Swain and Bal (2019) synthesized an IPN of Carrageenan-guar gum by microwave irradiation with unique features like thermal sensitivity at 48 °C and degradation above 200 °C. They reported micro rough surface morphology, hydrophilicity, and muco-adhesion properties with honey comb like internal architecture making it suitable for targeted drug delivery (Swain and Bal 2019).

Chen et al. developed an interpenetrating network of Konjac glucomannan (KGM)/kappa-carrageenan (KC) hydrogels, where KGM and borax react to form a chemical gel network and addition of KC component introduced the physical

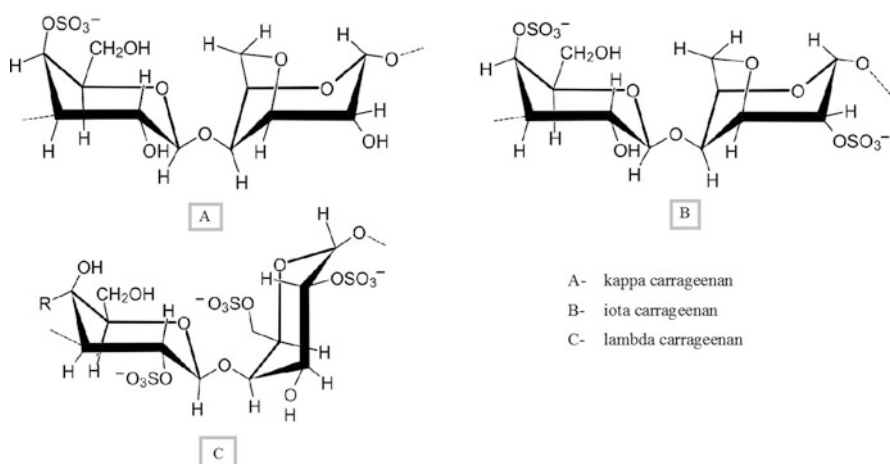


Fig. 8 Structural units of different types of carrageenan

network. The two networks formed are held together by hydrogen bonds formed between the organosulfate groups ($-\text{OSO}_3-$) present in the KC chains and hydroxyl groups ($-\text{OH}$) in the KGM chains. These IPN hydrogels are reported to exhibit very high tensile strength and is temperature sensitive and recoverable, with excellent self-healing performance (Chen et al. 2019).

Xanthan Gum-Based IPNs

Xanthan gum (XG) is another high molecular weight biopolymer produced by a gram-negative bacterium, *Xanthomonas campestris*, extra cellularly. It is a heterogeneous polysaccharide consisting of β -D-glucose units in the main chain which is linked by trisaccharides α -D-mannose, β -D-mannose, β -D-glucuronic acid in the side chains (Fig. 9). Xanthan gum has a net negative charge (anionic) contributed by the pyruvic acid and glucuronic acid groups in it (Shalviri et al. 2010). This polysaccharide polymer exhibits a lot of applications as thickener, emulsifier, suspending agent, and stabilizer for water-based systems, etc. (Talukdar and Kinget 1995).

IPN hydrogel of polymers based on xanthan gum and poly vinyl alcohol was synthesized as microspheres for the sustained release of drug, ciprofloxacin hydrochloride (Bhattacharya et al. 2013b). Hajikhani et al. produced semi-IPNs by acrylic acid/xanthan gum cross-linking in the presence of N, N'-hexane-1,6-dilbisprop-2-enamide, or 1,4-butandiol dimethacrylate as the cross-linking agent. XG-Poly acrylic acid semi-IPNs exhibited excessive adsorption potential and stability and is

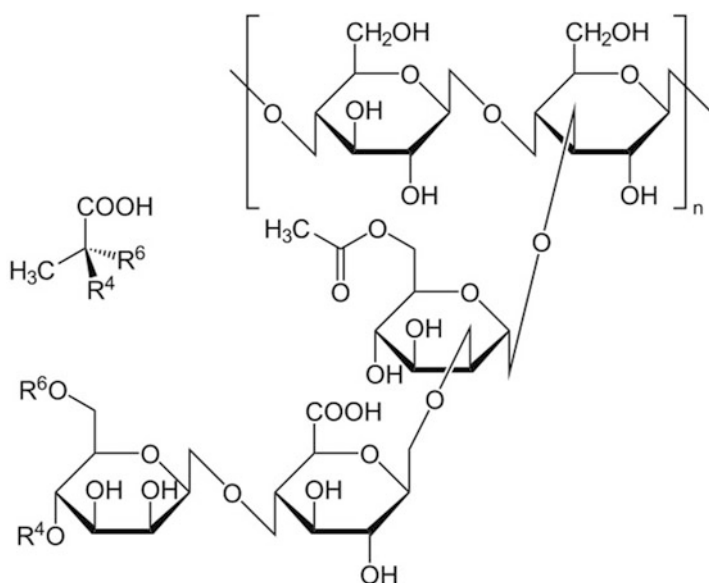


Fig. 9 Structural unit of xanthan gum

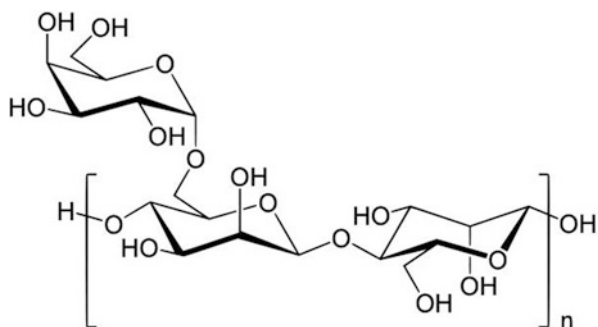
suggested as an environmentally benign and nontoxic material for application in drug delivery systems, wound healing, and dye removal (Hajikhani et al. 2019). Another group of scientists developed a xanthan gum/polyvinylpyrrolidone-co-poly acrylic acid hydrogels for controlled delivery of the drug, 5-fluorouracil to the colon region of the intestine. Another xanthan gum-based IPN fabricated for controlled drug delivery is a polymer of XG and polyvinylpyrrolidone (PVP) cross linked by acrylic acid monomers. This XG/PVP-copoly acrylic acid hydrogels used for drug delivery were cross-linked by ethylene glycol dimethacrylate and ammonium per sulfate/sodium hydrogen sulfite as a cross-linker and initiator (Anwar et al. 2021).

Guar Gum-Based IPNs

Guar gum is a biopolymer present in the seeds of guar or cluster bean (*Cyamopsis tetragonolobus* Linn.) in the endosperm region (Tripathy and Das 2013). It consists of repeating units of two 1,4-linked mannopyranosyl and one 1,6-linked galactopyranosyl groups (Fig. 10). The reaction of guar gum with borax produces borate ester bonds, which give the hydrogels formed from these with excellent self-healing properties (Li et al. 2018). In addition, the presence of borate ions makes the hydrogels conductive to electricity. Guar gum is used as an inexpensive and easily manageable carrier for long-lasting delivery of oral drugs (Varshosaz et al. 2006). A semi-IPN made of chitosan-guar gum with glutaraldehyde as cross-linking agent was used for regulated release of drug cefadroxil for almost 10 h by Reddy et al. (2006).

Recently, Cao et al. fabricated IPN hydrogels with guar gum and another natural polysaccharide polymer, gellan gum, by introducing borate and hydrogen bonds (Fig. 11). These IPN hydrogels with gellan gum, borax, and guar gum of appropriate concentrations show good tensile properties, excellent self-healing properties, and conductivity of electricity. The electric signals produced by these hydrogels during show good repeatability and reliability. The authors proposed the excellent mechanics, self-healing, and electrical conductivity of the novel polysaccharide-based IPN hydrogels can find use in human motion monitoring, intelligent wearable devices, etc. (Cao et al. 2021).

Fig. 10 Structural unit of guar gum



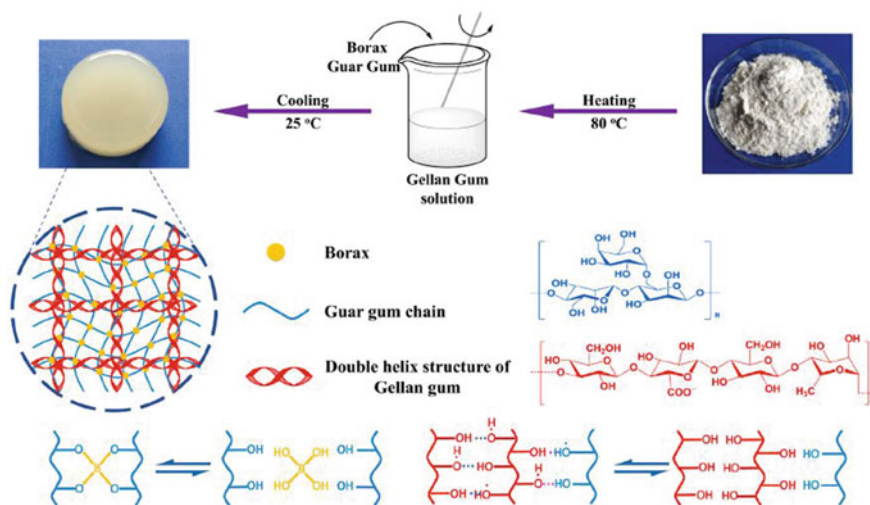


Fig. 11 Schematic illustration of the preparation process and structure of the guar gum/gellan gum IPN hydrogels. (Reprinted from Cao et al. (2021))

Hyaluronic Acid-Based IPNs

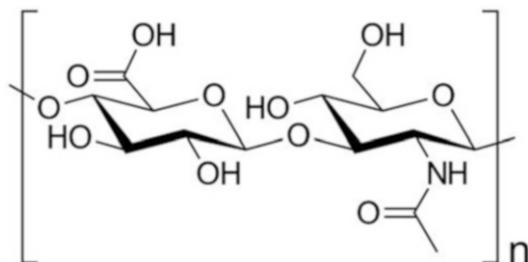
Hyaluronic acid (HA), frequently known as hyaluronan, is a linear high molecular weight polysaccharide having two alternating disaccharide units of β -1,4-D-glucuronic acid and β -1,3-N-acetyl-D-glucosamine (Kim et al. 2004) (Fig. 12). It exists as polyanion and is most commonly utilized in tissue engineering scaffold assembly and as component of implants. The chemical modifications, processing, polymerization, and bio-applications of HA have been deeply investigated and are used for many applications like ophthalmic surgery, arthritis treatment, scaffolds for wound healing, dermal filling, and tissue augmentation 8–9. The biocompatibility, biodegradability, and versatility make HA-based materials a favorite choice in tissue engineering as scaffolds. But its water solubility makes HA a less stable material and this can be effectively overcome by using other natural or synthetic polymers to fabricate HA-based IPN. Some examples are semi-IPN hydrogels composed of HA and synthetic polymers (Agostino et al. 2012) or natural polymers like HEMA derivatized dextran (Pescosolido et al. 2011b) and collagen (Chen et al. 2020a).

Locust Bean Gum-Based IPNs

Locust bean gum is a branch polysaccharide obtained from the seeds of carob tree (*Ceratonia siliqua*).

It consists of (1,4)-linked β -D-mannopyranose backbone with α -D-galactose (1,6-linked)-D-galactopyranose in the branching at the sixth position of the backbone (Glicksman 1963). Due to its nonionic nature, it is not affected by changes in

Fig. 12 Structural unit of hyaluronic acid



ionic strength. It can be dispersed in water to form a solution of pH 5.4–7.0. This solution can be converted to a gel by adding small amounts of sodium borate. Kaity et al. developed a unique IPN microsphere of locust bean gum and poly (vinyl alcohol) for controlled release of buflomedil hydrochloride orally (Kaity et al. 2013; Kaity and Ghosh 2016). In another study, ionotropic gelation with Al^{3+} ions was explored for fabricating IPN network of etherified locust bean gum and sodium alginate for application in drug release studies. These IPNs have better mechanical strength than homopolymer network and showed high efficiency for entrapping drugs and its sustained release (Dey et al. 2013).

Protein-Based IPNs

Protein biopolymers exhibit many desirable properties such as sustainable nature and its compatibility in body along with good mechanical strength. Various polymer structures like composites, gels, films can be made using these for applications like deliverance of drugs, regeneration of tissues, storage of bioactive compounds like vaccines, antibodies. Many hydrogels used for biomedical applications are fabricated with naturally derived protein biopolymers. Nevertheless, these hydrogels show poor mechanical properties. This problem can be encountered by developing interpenetrating polymer networks by combining natural biopolymers with other natural or synthetic polymers.

Various proteins were used in the preparation of IPN-based hydrogels in combination with either synthetic polymers or with gelatin, the protein derived from collagen. Usually this combination is used to increase the biocompatibility of the IPN hydrogels (Jain et al. 2009) and biological activity of synthetic polymers (Fathi et al. 2013; Singh et al. 2007) or to increase the structural stability of the gelatin nanofibers (Aduba et al. 2013). If the goal is to increase the stability of gelatin, polyethylene glycol diacrylate (PEG-DA) was used as a cross-linker during IPN synthesis. PEG-DA does not interact directly with gelatin rather it undergoes free radical cross-linking polymerization without reacting with the functional groups in gelatin. This results in a matrix with enhanced structural stability in aqueous solutions (Aduba et al. 2013). Cryogelation techniques used to fabricate macro-porous IPN composite hydrogels. Full interpenetrating network of polyacrylonitrile

(PAN)-gelatin was synthesized by Jain et al. with varied gelatin concentrations. The PAN-gelatin IPN is macroporous in nature and exhibits good flow rate and swelling efficiency. Hence, these IPNs have good interconnectivity of pores and convective flow within the network and hence can support cell growth within the scaffolds (Jain et al. 2009).

In a recent study, Chen et al. prepared an IPN of alginate/gelatin hydrogels in which cells can be encapsulated for in vitro three-dimensional cultures and organ bioprinting (Fig. 13). A double cross-linking model using calcium ions and transglutaminase to cross-link alginate and gelatin molecules, respectively, was carried out to improve the physiochemical properties and biochemical properties of the alginate/gelatin hydrogels. By altering the cross-linking and concentration of polymer, the properties of these hydrogels can be tuned to suit various applications. The cellular compatibility of the hydrogels was confirmed by in vitro cultures and bioprinting. These hydrogel is considered as a promising candidate for a range of biomedical applications like bioartificial organ manufacturing, high-throughput drug screening, and pathological mechanism analyses (Chen et al. 2020b).

Silk fibroin (SF), another biopolymer widely used in the preparation of IPN hydrogels, is a fibrous protein of silk fiber consisting of heavy and light chain polypeptides, linked by a disulfide link (Gil and Hudson 2007). The regenerated fiber has been considered as candidate for biomaterials owing to its good mechanical strength in the wet state, biocompatibility for the growth of cells, and high resistance against enzymatic degradation. It is also used to lessen the limitations of hydrogels

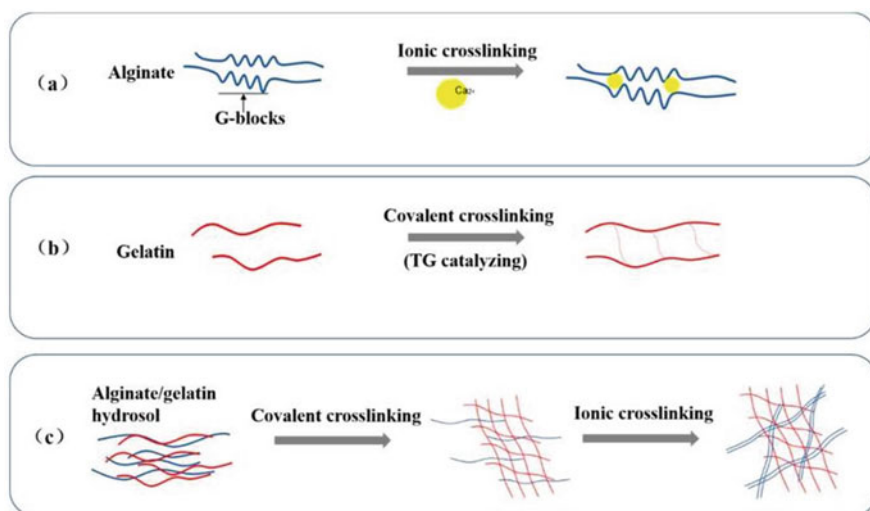


Fig. 13 Schematic description of alginate and gelatin cross-linking in alginate/gelatin IPN. “(a) G-blocks in two alginate chains are chemically (i.e., ionic) crosslinked by Ca^{2+} . (b) Transglutaminase (TG) catalyzed covalent linkages between two gelatin molecules. (c) An interpenetrating network in an alginate/gelatin hydrogel formed through both TG covalent and Ca^{2+} ionic crosslinks.” (Adapted from Chen et al. (2020b))

composed of Poly (N-isopropylacrylamide/PNIPAAm), like lack of biocompatibility, deswelling rate, and mechanical properties. Gil and Hudson (2007) produced IPNs of PNIPAAm and SF to improve the deswelling properties and suppressing the skin layer formation. IPN composite hydrogels composed of SF and other synthetic polymers were also developed by many researchers (Kundu et al. 2012; Mandal et al. 2009) and are of interest in tissue engineering and regenerative medicine.

The excellent biocompatibility makes silk fibroin hydrogel a perfect choice as biomaterial matrix, but the reduced transparency and flexibility limits its applications. This can be addressed by fabricating IPN hydrogels with changing ratios of silk fibroin/N-Vinyl-2-pyrrolidone mixtures cross-linked by H_2O_2 and horseradish peroxidase. These IPN showed high light transmittance capability and elasticity (Kuang et al. 2018). Silk sericin is a water-soluble globular protein derived from silk-worm, which has been used by Wu et al. in the preparation of IPN hydrogels with PNIPAAm (Wu et al. 2006) and PMMA (Wu and Wang 2010).

Fibrin is another biopolymer which exhibits excellent potential for tissue regeneration and wound healing because it can act as a backbone to promote cell adhesion, migration, and proliferation. Fibrin-based hydrogels extensively used in tissue engineering have inadequate mechanical performance, especially at the physiological concentration (2–5 mg/mL) where it quickly degrade, thereby limiting its clinical applications. Several strategies have been used in order to improve its mechanical properties by alteration of various polymerization parameters, but the approach of developing fibrin-based hydrogels IPN by combining fibrin networks with natural or synthetic networks is found to be very useful (Eyrich et al. 2007). A lot of studies reported IPN fabrication using fibrin and other polymers. Lee et al. made a hydrogel with enhanced mechanical strength using a combination of fibrin and hyaluronic acid (Lee and Kurisawa 2013). Shikanov et al. designed an IPN for culturing ovarian follicle by combining fibrin and alginate (Shikanov et al. 2009). Rowe and Stegemann developed IPN of enhanced for mechanical strength for cardio tissue engineering by combining fibrin and collagen (Rowe and Stegemann 2006). These are few systems where fibrin is combined with other naturally derived polymers. The combination of fibrin with synthetic polymers like polyethylene oxide (PEO), polyethylene glycol (PEG), and polyvinyl alcohol (PVA) allow greater flexibility to modify the physical properties and biomedical applications of fibrin-containing IPNs. Other protein-based IPN hydrogels are composed of fibrin and PEG (Akpalo et al. 2011), soy protein and PNIPAAm (Liu and Cui 2011a, b), collagen and hyaluronic acid (Suri and Schmidt 2009), and PAAm and poly (c-glutamic acid) (Rodríguez-Félix et al. 2011).

Other Biopolymer IPNs

In addition to polysaccharides and proteins, different vegetable oils are also used in fabricating IPNs by various groups. Bio-based polymers like poly (3-hydroxy-alkanoate)s (PHAs), synthesized by some microorganisms during stress conditions, provide an environmental-friendly alternative to synthetic polyesters used in

plastics. PHAs are suggested to have high potential for environmental, medical, or pharmaceutical applications such as drug delivery systems and tissue engineering due to its biodegradable and biocompatible properties. Still, few of the well-studied PHA's like poly (3-hydroxybutyrate) PHB and poly(3-hydroxybutyrate-co-hydroxyvalerate) PHBHV are brittle due to its relatively high degree of crystallinity. Thus, the thermal stability and processability of these compounds can be enhanced by cross-linking with vegetable oils to form semi-IPNs. The use of natural oils without any chemical modifications has several advantages such as renewability, availability, versatility, and the presence of reactive unsaturated groups. Carine Mangeon et al. developed a semi-interpenetrating systems based on sunflower oil and trithiol networks in which PHBHV chains are embedded thereby exhibiting a toughening improvement of such polyesters (Fig. 14). These novel cross-linked networks are formed inside the amorphous zones of the matrix, making it possible to significantly increase the elongation at break. Even though this effect is comparable to the plasticizing phenomenon, this also holds the advantage that the networks do not migrate over time and hence can act as long-lasting reinforcements of the matrix. This material also exhibits loss of thermomechanical properties when the cross-linked networks reaches 45% due to phase segregation (Carine Mangeon et al. 2018).

Another group fabricated a two-component IPN of modified castor oil-based polyurethane (PU) and polystyrene (PS) by the sequential method. The modification of castor oil was done by transesterification using triethanolamine to get transesterified castor oil (TCO). The polyurethane network was prepared from TCO using isophorone diisocyanate (IPDI) with dibutyltindilaurate (DBTDL) as catalyst. Concurrently, benzoyl peroxide (BPO) and *N,N'*-Dimehtylaniline were added to styrene as initiator and co-initiator, respectively. Diallyl phthalate was added as a cross-linking agent to form IPN and finally cast into films. These films were characterized by FT-IR, dynamic mechanical analysis, and thermogravimetry

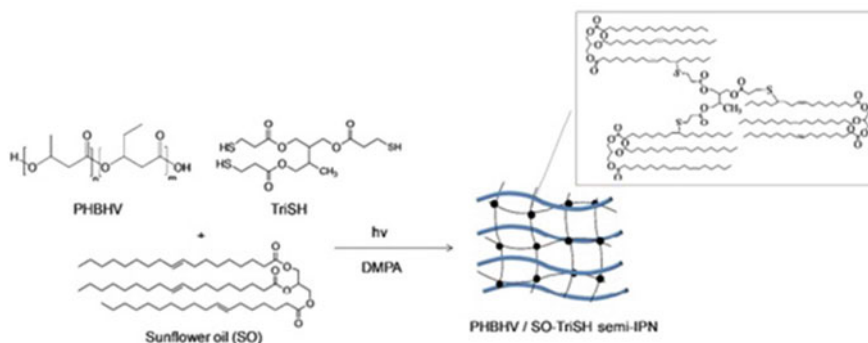


Fig. 14 Synthesis of poly(3-hydroxybutyrate-co-hydroxyvalerate)/sunflower oil and trimethylolpropane tris(3-mercaptopropionate) (TriSH) semi-IPN. (Reprinted (adapted) with permission from Mangeon et al. (2018). Copyright 2018 American Chemical Society)

analysis, and morphology was measured by scanning electron microscopy (Dave and Patel 2017).

Sequential interpenetrating polymer networks were prepared using polyurethane (PUR) synthesized from canola oil-based polyol with terminal primary functional groups and poly(methyl methacrylate) (PMMA). The morphology of the IPNs was investigated using scanning electron microscopy (SEM), modulated differential scanning calorimetry (MDSC), and a five-phase morphology, that is, sol phase, PUR-rich phase, PUR-rich interphase, PMMA-rich interphase, and PMMA-rich phase, and was observed for the IPNs from MDSC. The mechanical properties of the IPNs were observed to be superior to those of the constituent polymers due to the finely divided rubber and plastic combination structures in these IPNs (Kong and Narine 2008).

Applications of IPNs

Some of the inherent properties of biopolymer-based IPNs made them a highly desirable polymer for many applications. They are (a) the biocompatibility/biodegradability, (b) increased mechanical strength and phase stability than its components alone, (c) synergism of individual component properties to produce networks of better features, (d) complete cross-linking/blending and hence thermodynamic incompatibility and phase separation between the component polymers is not usually observed, (e) ability of IPN to keep the phases separate when subjected to stress, and (f) ability for controlled release of drug delivery.

IPNs find its application in many areas like biomedical applications, optical applications, fuel cell applications, coating and adhesives, adsorption of gases, sound and vibration damping, metal ion removal, solar heating, and thermal control applications (Raveendran and Sharma 2016). Among these, biopolymer-based IPNs are extensively studied for its biomedical applications.

Biomedical Applications of IPN

Interpenetrating polymer networks based on biopolymers are investigated with keen interest for its potential applications in biomedical field due to its biocompatibility and biodegradability in addition to other physiochemical factors. Many studies established the excellent capability of various biopolymer IPNs as excipients for sustained and controlled drug delivery, scaffolds in tissue engineering, for gene delivery and other applications.

IPN-Based Drug Delivery System

IPNs of different forms like films, hydrogels, tablets, capsules, microspheres, sheets, sponges, matrix are extensively used for drug delivery. The most important

requirements for an effective drug delivery system are the effective control of drug release and stability of the excipient. IPNs can be used for delivering drugs in a time- and rate-specific manner, and to achieve this, chemical modification with addition of functional groups in the polymeric excipients is usually done (Twaites et al. 2005) without causing issues of limits of toxicity and compatibility. IPN-based drug delivery systems are used to deliver the drug at a definite rate for required period of time. The properties of IPN like enhanced solubility of hydrophobic drugs, good swelling capacity, stable drug formulations, biodegradability, biocompatibility, weak antigenicity, and specific tissue targeting make them suitable for drug delivery applications. The various forms of IPN used for drug delivery are discussed below.

Hydrogel

Smart biomaterial in the form of hydrogel formulation has attained a lot of interest in tissue engineering and drug delivery owing to its physicochemical properties. Hydrogels are cross-linked or physically entangled three-dimensional polymeric networks of hydrophilic nature, which exhibits remarkable swelling capacity (Peppas et al. 2000). The properties of hydrogels can be improved by fabricating IPNs hydrogels, which offer the possibility for a range of physical formulations like microspheres, nanoparticles, and films. In IPN hydrogel-based drug delivery, an exceptional advancement happened with the development of smart drug delivery systems (SDDS), also called stimuli-sensitive delivery systems. The idea of SDDS is based on the conversation of physical and chemical properties of polymer systems by an environmental stimulus, which can be physical stimulus (temperature, electricity, light, and mechanical stress), chemical stimulus (pH and ionic strength), or biological signals (enzymes). Such stimuli can be an internal signal from the body of the living organism where the drug is delivered or external signals which are artificially induced. This nature of hydrogels has made them a polymer of great interest as drug delivery vehicle capable of controlling drug release and drug targeting.

Targeted drug delivery in specific site is achieved by the formulation of pH-sensitive IPN hydrogel beads of ibuprofen, which reduces the release of drug in acidic medium and controls the drug release in alkaline medium (phosphate buffer) depending on the need (Ray et al. 2011). Another team of researchers developed IPN hydrogels of calcium alginate and dextran hydroxyethyl-methacrylate and examined its potential for sustained protein release and chondrocytes (cartilage cells) encapsulation capability. It was found to be a promising system for protein delivery and tissue engineering applications (Pescosolido et al. 2011a).

A soft hydrogel IPN of silicone elastomers with a poly(2-hydroxyethyl methacrylate) (PHEMA) in the range of 13–38% (w/w) was synthesized by using carbon dioxide-based solvent mixtures under high pressure. The IPN material was loaded with the antibiotic ciprofloxacin, and the resulting drug release was shown to inhibit bacterial growth when placed on agar, thus demonstrating the potential of this IPN material for future applications in drug-releasing medical devices (Steffensen et al. 2016).

Shin et al. developed an interpenetrating network of polyacrylic acid (PAA) and polyacrylamide (PAAm) that forms hydrogels with positive thermoresponse, that is,

drug release increases with increasing temperature. These IPN hydrogels swell above its upper critical solution temperature, UCST, due to the disruption of hydrogen bonding between two different networks at higher temperatures, indicating the possibility of enhanced drug release with increase in temperature (Shin et al. 1997). Another work on the same IPN with grafted β -cyclodextrin showed a faster thermoresponse and lower UCST (35 °C) and a lowered effect of salt on the swelling. Incorporation of a model drug, ibuprofen, showed a positive drug release with a controlled rate above and below the UCST (Wang et al. 2009). Semi-interpenetrating networks synthesized by Mundargi et al. formed by cross-linking gellan gum microspheres with interpenetrating poly (*N*-isopropylacrylamide) chains allowed pulsatile drug release below the lower critical solution temperature due to swelling of the spheres and greater water transport through the spheres (Mundargi et al. 2010).

Microspheres

Microspheres, the free flowing tiny spherical powder of natural or synthetic polymers of 1–1000 μm size (Lohani and Gangwar 2012), constitute another important IPN-based drug delivery system. IPN microspheres are regarded as a multipurpose carrier for drug targeting and release due to its capability to entrap drugs without losing its pharmacological properties, its compatibility and degradability in biological systems, bioavailability, and sustained release characteristics. In vitro studies to analyze drug release were carried out by many research groups to identify drug delivery properties of IPN microspheres. Xanthan gum facilitated superabsorbent polymeric microspheres was effectively used for sustained release of ciprofloxacin hydrochloride (Bhattacharya et al. 2013a). Anticancer drug like 5-fluorouracil encapsulated in carbohydrate grafted IPN microspheres were found to have enhanced drug release and bioavailability properties (Sekhar et al. 2012).

Tablets

IPN-based tablets are also prepared by many groups for facilitating sustained release. Calcium ion cross-linked IPN matrix tablets of polyacrylamide-grafted-sodium alginate were prepared for sustained release of diltiazem hydrochloride. The degree of swelling capacity of IPN matrix and viscosity of the gel formed following dissolution of the polymers governs the drug release from the matrix (Mandal et al. 2010). IPN tablets prepared by a wet granulation/covalent cross-linking method matrices were found to be effective for sustained drug release of antihypertensive drug (propranolol hydrochloride) (Kulkarni et al. 2013).

Films

IPN-based films prepared by blending natural polymer collagen and synthetic polymer polyvinyl alcohol were loaded with growth hormone to investigate its potential as a drug delivery system and found that the release of growth hormone is dependent on both initial loading concentration of drug and the content of each polymer in the IPN (Cascone et al. 1995).

Sheet

Sheeting is another method of designing IPN-based drug delivery system. These are mainly used in various types of wound dressings and scar management products. An IPN composed of polymeric material like polyol (allyl carbonate), for example, nouryset[®]200 and epoxy resin, is developed by 70–95 parts by weight of polyol (allyl carbonate) by means of radical initiation and polymerizing partially or completely concurrently is an epoxy resin forming mixture composed of 10–90 weight % of aliphatic or cycloaliphatic epoxide and 90–10 weight % of polyol/anhydride adduct (Dillon 2006).

IPN in Tissue Engineering

Tissue engineering plays an important role in facilitating the repair and regeneration of diseased tissues, and hence, the development of tissue engineering scaffolds has emerged as a topic of great interest in biomedical research. Hydrogels are a potent candidate for scaffold formulation, but control of porosity and the micro-architectural features in hydrogels is a challenging task. These issues can be effectively overcome by designing hydrogel IPNs. Production of the hydrogel IPNs received much attention because it improves the mechanical properties, and more importantly, it mimics the extracellular matrix which can be used as cell culturing scaffolds for tissue engineering. Many synthetic polymers are used for fabricating IPN hydrogels, but it is not preferred for tissue engineering due to its nondegradable nature.

Biologically derived IPNs from collagen and hyaluronic acid have been widely explored for tissue engineering applications. Natural biomaterials-based IPNs possess active cell-responsive mechanical properties that facilitate suitable conditions for tissue growth. For example, an IPN of fibrin and alginate was reported to have mechanical properties for ovarian follicle culture in vitro (Shikanov et al. 2009).

IPN gels are also prepared by separated procedures, in which primal networks, followed by other networks, are formed by adding chemical reagents or subjecting to external stimuli. Recently the study by Ishikawa et al. designed a one-pot and in situ gelation system, which involved strategic selection of precursors for constructing IPN gels by simply mixing them (Fig. 15). The design involved two types of gelation processes: RADA16 peptide self-assembling and covalent bond formation between chitosan (CH) and *N*-hydroxysuccinimide ester-terminated poly(ethylene glycol) (NHS–PEG–NHS). Then they cultured chondrocytes in CH/PEG/RADA16 and showed that construction of the IPN structure promoted the embedded chondrocyte properties for the formation of the articular cartilage. They also reported a lower inflammation and higher protein production in mice implanted with CH/PEG/RADA16-containing chondrocytes than in those with clinically used atelocollagen gel, appealing the feasibility of the proposed IPN hydrogel design for use as cell culturing scaffolds in tissue regeneration (Ishikawa et al. 2020).

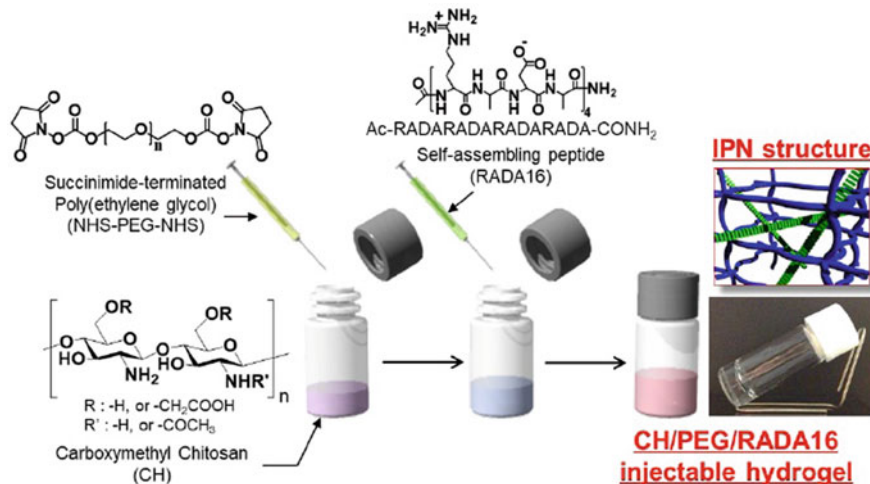


Fig. 15 Schematic illustration of “one-pot” synthesis of the CH/PEG/RADA16 injectable hydrogel with interpenetrating polymer network (IPN). (Reprinted (adapted) from Ishikawa et al. (2020). Copyright (2020) American Chemical Society)

Hago and Li developed a polyvinyl alcohol/gelatin (PVA/GE) IPN hydrogels by a combination of enzymatic and cyclic freezing-thawing method. The IPN was prepared by cross-linking of various concentration gelatin in the presence of transglutaminase enzyme by using the freezing-thawing cycles technique. Their study observed that these hydrogels exhibited high fluid absorbing ability, which makes it a suitable choice to treat exudative wounds. The study on proliferative capability of fibroblast cell lines seeded on the IPN hydrogel showed an increased cell viability and proliferation (Hago and Li 2013).

IPN in Gene Delivery

Cationic polymers are the favorite matrices for gene delivery because of their ability to cover the anionic nature of DNA, which can, in turn, lead to efficient transfection (De Smedt et al. 2000). A good gene delivery system should exhibit features like resistance to premature enzymatic degradation and aggregation, target-specific uptake of gene, and controlled release of the genetic material. Semi-IPNs synthesized by dispersing quaternary ammonium salt hydrogels into neutral monomers such as acrylamide or 2-hydroxyethyl methacrylate exhibited gene delivery potential (Sahiner et al. 2006).

IPN in Ophthalmic Applications

IPNs are used to as ophthalmic biomaterial especially as corneal substitute (artificial corneas) for treating blind patients with corneal disease. Glucose-permeable poly

(dimethyl siloxane)–poly(*N*-isopropyl acrylamide) IPNs were proved as a potent ophthalmic biomaterial. Collagen–phosphorylcholine IPN hydrogels were also designed as corneal substitutes to retain the enzymatic resistance, hydrophilicity, and increased mechanical strength (Liu and Sheardown 2005).

Food Packaging Applications of IPN

Plastics, owing to its low cost, water and dirt resistance, favorable mechanical, thermal, and optical properties, are commonly used material of choice for packaging purposes. But the environmental issues caused by plastics prompted researchers to find a sustainable alternative and nowadays, interpenetrating hydrogels are considered a suitable alternative for conventional plastics in packaging industry. Using interpenetrating hydrogels allows the opportunity to generate hydrogels from bio-based materials to develop biodegradable packaging materials. Cellulose interpenetrating hydrogels show considerable significance in biodegradable food packaging. Cellulose-based hydrogels, consisting of cellulose and its derivatives, can be blended with gelatin, polyvinyl pyrrolidone, or polyvinyl alcohol to offer convenient packaging food materials. Introduction of silver nanoparticles and antioxidants can augment the properties of these hydrogel sheets (Amini et al. 2016). Composites based on poly (vinyl alcohol) and micro- and nano-fibrillated cellulose were also prepared. Hydroxyethyl methacrylate can be introduced with photo-initiator helped producing an IPN with improved distribution and interfacial adhesion, which are beneficial for packaging applications (Bai et al. 2015).

Other General Applications of IPN

Due to its unique properties, the applications of IPNs find wide applications in various areas. Most of the applications in fields other than biomedical area mainly exploit the use of synthetic polymers than biopolymers. These wide spread applications are mainly due to its flexibility, controlled phase domain sizes, thermosetting properties, energy absorption potential, etc. Some of the key areas where synthetic polymer-based IPNs are used include (i) selective transportation of liquids and gases for applications in protective coatings, packaging, etc. (George and Thomas 2001), (ii) ion exchange membranes for laboratory use and large-scale applications like effluent treatment, sea water desalination, and concentration or separation of pharmaceutical and food (Xu 2005), (iii) removal of metal ions by designing chelating adsorbing membranes using polymers (Metayer and M'Bareck 1997), (iv) sound and vibration damping properties for applications in automotive, aircraft, construction, and appliance industries (Aklonis and MacKnight 1983), (v) fuel cells and solar cells (Wu et al. 2007), (vi) to improve properties of elastomers, and (vii) in energy, process, aeronautics, and many other applications (Singh 1998).

Conclusion

Biopolymer-based interpenetrating polymer networks is one of the rapidly expanding fields in polymer science with immense applications. Due to the favorable attributes offered by the constituent polymers and the novel properties of the cross-linked network, IPN exhibits unique features, which hold high industrial significance. The value of the cross-linked interpenetrating polymer networks as a bio-compatible biomaterial is immensely explored in the areas of tissue engineering and drug delivery. IPNs are revolutionizing the field of biomaterial science starting from the development of bioengineering tissues, such as bone substitutes, tissue, and cartilage scaffolds to targeted drug and gene delivery. Biopolymer-based IPNs are considered as a sustainable alternative in various fields and hence are highly investigated by polymer scientists. The immense potential that lies in these polymer networks needs to be fine-tuned and explored more deeply to make commercially viable/affordable products in a large scale.

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Antony V. Samrot, Shree Krithika Sivasuriyan, Sneha Xavier,
Nagarajan Shobana, Deenadhayan Rajalakshmi,
Mahendran Sathiyasree, and Sanjay Preeth Ram Singh

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Abstract

A range of applications benefit from the properties of biopolymers, which are organic materials produced by living cells. These properties include biocompatibility, greater stiffness, toughness, flexibility, electrical conductivity, and biodegradability. The applications include edible films, transport materials, biomedicine,

A. V. Samrot (✉)

School of Bioscience; Faculty of Medicine, Bioscience and Nursing, MAHSA University, Jenjarom, Selangor, Malaysia

S. K. Sivasuriyan · S. Xavier · N. Shobana · D. Rajalakshmi · M. Sathiyasree · S. P. Ram Singh
Department of Biotechnology, School of Bio and Chemical Engineering Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India

packaging materials for the food industry, emulsions, medication medical implants such wound healing, artificial organs, tissue engineering, and dressing. In this review, commonly known biopolymer-based gels, mechanism of its gelation, applications, and its advantages and disadvantages are briefly explained.

Keywords

Biopolymer · Agarose · Polysaccharide · Drug delivery

Introduction

Biopolymer gels have piqued scholars and their practical curiosity for a long time. Industrial interest, particularly from the food industry, has never been higher than it is currently due to the recent need for low-fat products (Clark 1996). Many biopolymers that can form a gel in aqueous solution, such as agarose, carrageenan, and gelatin, all have one thing in common: the gelation is preceded by a shift from a disordered to an ordered conformation (Viebke et al. 1994). These systems are limited to basically physical gels produced in an aqueous environment from biopolymeric materials (Clark 1991). Hydrocolloid interactions in aqueous environments have been extensively investigated with respect to their thermodynamic compatibility and interbiopolymer complexing (Zasytkin et al. 1997). Biopolymers are made using thermally driven gelation, addition of new component to the start solution, and prepared from the existing gel (Clark 1991). These biopolymers are implicated in various drug delivery applications, antibacterial activity, wastewater treatment, etc. (Samrot et al. 2019, 2020a, b, 2021a, b, c; Chellapan et al. 2018; Justin et al. 2018; Shobana et al. 2019, 2022; Samrot and Sean 2022; Abirami et al. 2021).

Gel Derived from Biopolymer

Natural Polymer: Agarose Gel

Agarose is a polysaccharide derived from the red algae named *Rhodophyceae* (Weinberger 2000). It is composed of nearly 400 repeating units of agarobiose linked together by 1→3 D-galactopyranose and 1→4 3,6-anhydro-α-L-galactopyranose with an average molecular weight of 120,000 Da (Roberts and Martens 2016; Subramanian and Varade 2017). The gelation occurs by dissolving agarose in boiling water as it is insoluble in water and when it is cooled at temperature below 45 °C hydrogen bonds form between the chains (Gustavsson and Son 2003; Mayer and Fiechter 2013). The gelling temperature depends on the concentration of the solution, composition of monomers, and method of derivatives of agarose such as addition of de-structuring salts or by hydroxyethyl derivatization (Medin 1996). Agarose gel is widely used in cartilages, tissue engineering, etc for the encapsulation of cells (Roberts and Martens 2016).



Fig. 1 Application of agarose from seaweed

Biopolymer Gel: Alginates

Alginates are the natural anionic polysaccharides derived from brown algae such as *Macrocystis pyrifera*, *Laminaria hyperborea*, *Ascophyllum nodosum*, and from bacteria such as *Azotobacter* and *Pseudomonas* (Szekalska et al. 2016). It comprises of 1, 4 β -D-mannuronic acid (M block) and 1, 4 α -L-guluronic acid (G block) which are arranged either in homogeneous or heterogenous block-like pattern (Szekalska et al. 2016). Alginate biopolymers are precipitated by dissolving monovalent alginate salts in alginic acid maintained at the pH of 3.38–3.65 (Abasalizadeh et al. 2020). Two types of methods exist for the process of forming gels from alginate which includes ionic cross-linking using cations and acid precipitation (Ching et al. 2015). Alginates are capable of forming gel with multivalent, divalent, and trivalent cations through cross-linking (Ching et al. 2015). The properties of alginate gel such as high water absorbing capacity, porosity, biocompatibility, and permeability makes them an ideal candidate for wound dressing, treatment for cancer, 3D bioprinting, etc. (Abasalizadeh et al. 2020) (Fig. 1).

Collagen-Based Polymer

Collagen is responsible for holding together the body's conjunctive and connective tissues, including skin, joints, and bones. It is a fibrillar protein, a component of extracellular matrix found abundant in most of the living organisms (Avila Rodríguez et al. 2017). The structure of collagen consists of a triple helical region of Gly-x-y motifs where X is proline and Y is hydroxyproline (Gómez-Guillén et al. 2011). There are almost two different forms of collagen, however the ones that are most frequently seen are types I, II, III, and IV (Seth et al. 2018). The sources of collagen include *Althaea cannabina*, *Staphylococcus carnosus*, *Rhopilema asamushi*, *Stomolophus meleagris*, *Catostylus tagi*, and *Rhizostoma pulmo* etc. (Sionkowska et al. 2020). Pro-collagen is produced first, followed by the disintegration of propeptides utilising the enzymes C-proteinase and N-proteinase, respectively, and finally the cross-linking of fibrils (Sionkowska et al. 2020). It is used as the major component in the pharmaceutical industries as it acts as moisturizer and a humectant (Sionkowska et al. 2020) (Fig. 2).



Fig. 2 Production of gelatin powder into collagen

Polymer Derived from Gelatin

Gelatin is a type of protein obtained from collagen by partial hydrolysis present in the skin and bones either in acidic or alkaline conditions (Narayanaswamy et al. 2016; Deshmukh et al. 2017; Mohiti-Asli and Lobo 2016). It is translucent, colorless and tasteless powder consisting of residues like glycine, proline, and 4-hydroxy proline. Gelatin form into gels at a temperature of 35 °C due to the formation of interchain hydrogen bonds, which are transparent, elastic, and permeable to oxygen (Deshmukh et al. 2017). It is widely used in the application of tissue engineering, food industry, pharmaceutical industry, cosmetic industry, photography, and other fields due to the properties such as cell adhesion, high biocompatibility and water solubility, flexibility, adhesiveness, growth, cost-effectiveness, and low immunogenicity as well as the capacity to generate transparent gels under certain conditions (Van Vlierberghe et al. 2014; Deshmukh et al. 2017; Alihosseini 2016).

Using Fibrin as Biopolymer

Fibrin is a protein derived from blood (i.e., fibrinogen and thrombin) (Catelas et al. 2008; Sundararaghavan and Burdick 2011). It is obtained from fibrinogen through the process of cryoprecipitation, ammonium sulfate precipitation, where thrombin degrades fibrinogen to form fibrin monomers, which link together by hydrogen bonding and becomes insoluble through the reaction of thrombin with plasma factor XIIIa (Burdick and Stevens 2005). The applications of fibrin include the formulation of foams, sheets, particle adhesives, and sealants, which are broadly used in encapsulation of cell, growth factor delivery, and in tissue engineering (Burdick and Stevens 2005; Sundararaghavan and Burdick 2011).

Biopolymer Gel: Hyaluronic Acid

Hyaluronic acid (hyaluronan, HA) is a disaccharide made up of (1→3)- β *N*-acetyl-D-glucosamine and (1→4)- β D-glucuronic acid with no sulfate bond, and belongs to a group of glycosaminoglycans (Kogan et al. 2007). It is a component of the extracellular matrix that is secreted by cells like fibroblasts and chondrocyte which play important roles in cell signaling and wound healing (Dovedytis et al. 2020). HA has been extensively used in drug delivery applications in the form of films, microspheres, liposomes, fibers, and hydrogels due to its biocompatibility, biodegradability, and easily changed chemical structure. It is also used in chronic wound repairing, cell migration, and skin healing (Tammi et al. 1989; Shaharudin and Aziz 2016; Litwiniuk et al. 2016).

Structure of Biopolymer Gels

Biopolymer gels tend to be soft solids, where the stress-strain relationship determines the its character and such materials typically do not rupture even after enduring considerable strain intervals under the condition of deformability. In terms of gelling biopolymers, the clustering of disordered molecules, and the association of aligned polymer chains, can be characterized as the mechanism of gelation to give a structural form of it. In addition to electrostatic interactions, ions may also play a role in bonding, and a cross-linking reaction can also be caused by hydrogen bonding between sections of peptide chains. When proteins aggregate, a densely branched cluster of filaments is formed. Through interparticle interaction, specific gels of varying degree of homogeneity can be formed (Clark et al. 1991).

Mechanism of Gelation

Gelation provides information about the structural aspects of biopolymer gels, as well as the mechanism of gel formation. The gelling biopolymer, such as gelatin, is formed by denaturing and hydrolyzing collagen, and sets into a transparent gel when left to cool below 40 °C. In terms of gelling properties, the formation of triple helices is evidently the leading method of cross-linking, since helix-helix cross-linking does not appear to be the primary mechanism in the formation of gels (Clark et al. 1991). In addition to forming physical junctions, chain entanglements can also provide the precursors for the formation of a three-dimensional network of polymer chains of gels and to permit gelation, hydrophilic, and hydrophobic interactions needed to reach a critical value, polymer ionization could be decreased to achieve this value (Montembault et al. 2005).

The molecules or segments of the solution have to be close enough to each other so that the attractive force can fix the structure of the solution. The formation will be a spontaneous reaction if Brownian motion or diffusion occurs. Despite this, the mobility of the molecules or segments may be severely limited due to the high viscosity and hydrogen bonding of the molecules, caused by the long chain and hydration of the sol (Maekaji 1974).

A cross-link is a bond that connects one polymer chain to another where it can be ionic or covalent. During the cross-linking process, a liquid polymer becomes a “solid” or a “gel” by reducing its mobility. In simple words, the chains of a liquid polymer can be linked together to form a solid or a gel. It has more molecular mass, so it is mechanically stronger and is resistant to heat, wear, and chemical attack. Cross-linked polymers are important because they provide resistance to heat, wear, and chemical attack (Maitra and Shukla 2014). This mechanism can be done in two properties such as chemical cross-linking and physical cross-linking.

Chemical Cross-Linking

The cross-linking of biopolymer gels can be accomplished by chain growth polymerization, addition and condensation polymerization, as well as by gamma or

electron beam polymerization. It includes free radical polymerization, controlled free radical polymerization, anionic polymerization, and cationic polymerization. During this process, a free radical active site is generated that adds monomers in a chain-like formation (Maitra and Shukla 2014). Gelation of biopolymer gels are produced by chemically cross-linking gelatin, where gelatin gels are not stable as the cross-linked network can be rapidly hydrolyzed by protease. Therefore, gelatin gel degradation can be accelerated or adjusted by introducing various chemical cross-links (Li et al. 2012).

Physical Cross-Linking

The process of making physically cross-linked hydrogels involves interactions between ions, crystallization, formation of stereocomplexes, hydrophobic polysaccharides, hydrophilic polymer chains, and hydrogen bonds with proteins (Maitra and Shukla 2014). The physical cross-linking of the structures of biopolymer gel defines the physical factors, such as pH, temperature, etc. The thermodynamics of cross-linking gelatin hydrogels are affected by the temperature of the solution. Aqueous gelatin solutions become aqueous gels when cooled below 30 °C. (Li et al. 2012). Under certain conditions, hydrogels can be cross-linked under physiological pH conditions at room temperature without the need for ionic groups in the polymer. Metallic ions which are naturally present in the hydrogel produce stronger bonds (Hennink and Nostrum 2002).

The Volume Phase Transition (VPT) in Polymer Gels

A volume phase transition (VPT) occurs when the degree of swelling changes “abruptly” (continuously), and when there exist a possibility of coexisting gel phases with differing swelling degrees. The phase equilibrium of swollen and cross-linked systems is unique to this type of system (Millar et al. 1965). When networks are forming, phase separation is a consideration that leads to the concept of volume phase transition. Macroporous cross-linked ion exchange resins became relevant in the early 1960s due to their combination of ion exchange and selective adsorption abilities (Millar 1960). The matrices were fabricated by cross-linking free radical copolymerization, diluted with both low-molecular-weight diluents and a soluble polymer, in the presence of additives. In the USA, there was someone working on practical problems (R. Kunin), but in one of the Czech research centers a broader focus was being developed (Seidl et al. 1967). In addition to evolving methodologies for gel permeation chromatography, research on ion-exchange matrix was focused on a variety of separation materials (Rosi et al. 2003). The formation of pores was analyzed empirically at first, but we were eager to integrate them into the polymer theories represented at that time by P. J. Flory Handbook (Flory 1953). Toward the incipient stage of phase separation, we considered the formation of heterogeneous structures as a thermodynamic process. Phase separation was characterized by the cross-link density (a function of the polymerization conversion) reaching a value of 12 such that the ultimate swell in the existing liquid (diluent plus monomer) occurred

($\varphi_2 = \varphi_0/2$) (Millar et al. 1965). The degrees of cross-linking, molar volume of the diluent, and dilution all contributed to improved phase separation. Phase separation became understandable for the first time when it became apparent that good solvents and soluble polymers with similar chemical compositions to network chains are so effective (Dušek and Sedláček 1969).

Studies of phase separation in gels provided further insights into why phase separation may occur in a microscopically or macroscopically organized form in some cases (Alberti et al. 2019). Approximations were made in regard to the evolution of structure, the role played by sol fraction, system dispersion, and interactions among the component systems. Analyzing the effect of various phase separation factors, it was discovered that the chemical potential of the dilute ($(\Delta\mu_1/RT)$ or its Maxwell construction form $(\Delta\mu_1/RT\varphi_2)$) passed through two extremes with respect to the polymer fraction (φ_2) passing through two extremes, which satisfy the condition $\Delta\mu_1 = 0$ for pure diluent. Using three-phase equilibrium, two gel phases of differing swelling levels and satisfying a particular condition could maintain an equilibrium between two phases of gel

$$\begin{aligned}\mu' \\ 1 &= \mu'' \\ 1 &= 0; \mu'/2 = \mu''\end{aligned}$$

Here, μ_2 (φ_2) is the equation determined by the Gibbs-Duhem equation or ΔG_{sw} showing two minima.

The function of phase separation in gels was also explained further by Alberti et al. who revealed that in some cases phase separation takes place on a micro- or a macrolevel. In order to model changes in structure, sol fraction, system dispersion, multicomponent interactions, etc., a number of approximations were adopted (Dušek and Patterson 1968). It was found that the adjustment of the chemical potential ($\Delta\mu_1/RT$) or its Maxwell construction form $(\Delta\mu_1/RT\varphi_2)$ for phase separation passed through two extremes where the roots satisfy the condition $\Delta\mu_1 = 0$ for pure diluent. In other words, it could be that two gel phases with differing degrees of swelling could achieve equilibrium and satisfy the condition of three-phase equilibrium. The Gibbs-Duhem equation or simply ΔG_{sw} , a two-minimum equation could be used to calculate φ_2 . The underlying phase diagrams for these systems show abrupt changes of the degree of swelling with temperature or other stimuli, which can be mapped out to the values of the chain concentrations, concentration coefficients, and Flory-Huggins interaction parameters (Dušek and Sedláček 1969).

Methods for Enhancing the Stability of Biopolymer Gels

Synthetic polymers are proving to be a natural mimic for biopolymers due to the enormous capacity and prospective applications (Jaspers et al. 2014). Poly-isocyanopeptides (PICs) are a class of artificial polymers with an attractive helical structure and a high helical inversion barrier (Yashima et al. 2016). A peptide

hydrogen bond network between n and $n + 4$ along the polymer spine stabilizes an $\sim 4_1$ (four repeats per turn) β helical conformation (Cornelissen et al. 2001). In addition, this hexagonal structure grants the polymer a high degree of stiffness (van Buul et al. 2013). Polyisocyanopeptide gels enhanced by ethylene glycol are similar in nearly all aspects to biologic gels and are more complex biomaterials (Jaspers et al. 2016). These gels display ultrasensitive mechanical response due to the formation of a network of flexible bundles when heated into the mesophilic region (37 °C). In addition, these gels decorated with GRGDS peptides can be used as three-dimensional scaffolds to manipulate stem cell differentiation (Das et al. 2015). The number of synthetic biomimetic polymers with high thermal stability is still relatively low.

Three adjacent alanine groups give TriPIC its exceptional stability. As the amide groups both run parallel to the polyisocyanide backbone, they create two parallel arrays of hydrogen bonds that stabilize the rigid, helical backbone of TriPIC (Yashima et al. 2016). A hydrophobic core surrounds the polymer backbone after the amide regions become partly inaccessible to water. The first hydrogen bond (A, not accessible to water) keeps stable as heat is applied, whereas the second bond (B), which is stretched, results in a more defined helix, and stiffer polymer (Van Buul et al. 2013). The hydrophobic interactions of the glycol tails drive the assembly of polymers once a gelation temperature has been reached. This induces an unusual thermal stability of the gels, which can remain stable for hours at 80 °C (Das et al. 2015).

Host-Guest Macromers (HGMS)

As they present a translucent appearance to HGM hydrogels, such heterogeneity can be correlated with the heterogeneous domains observed in chemical hydrogels prepared from UV cross-linked macromers (Waters et al. 2010). The hydrogels of HGM reflect this heterogeneity in the way they appear translucent. Studying the nanostructure of HGM hydrogels using Small-Angle X-ray Scattering (SAXS) and Transmission Electron Microscopy (TEM) is one of our main research objectives (Xia et al. 2013). Since the AD functionalization of HA is not position dependent, the entire heterogeneous structure of the nanoclusters is formed during UV-induced polymerization of the HGMS. Based on this data, a SAXS analysis found broad correlation peaks within the q (covariance matrix, $q = [4\pi/\lambda] \sin \theta$) range, from 0.4 to 1 nm⁻¹. The large peaks are due to scattering centers that are distributed randomly in the matrix of the hydrogel. These peaks are influenced by the AdxHA modification degree. With more modification degrees, the peak is located closer to $q = 1$. The spacing between host and guest nanoclusters can be estimated using Bragg's law (Eq. 1) $\lambda \theta \pi d = 2 \sin q$ 2 max, where d equals the distance between scattering centers and q_{max} is the peak position (Alberts et al. 2002).

Compressive Properties of the HGM Hydrogels

In addition, host-guest interactions are reversible, which means that they can easily be rearranged and regrouped upon compression. Therefore, the compressible HGM

hydrogels are much stronger and softer than the chemical MeHA hydrogels. MEHA hydrogel ruptures at stress levels below 50%. Conversely, the HGM hydrogels (4 wt% AdxHA) can withstand substantially more strain, up to about 80% (Zhao et al. 2010).

Host-Guest Macromer Hydrogels for Cartilage Regeneration

Human mesenchymal stem cells (hMSCs) can differentiate into several mesenchymal lineages, including cartilage, and our HGM hydrogels are demonstrated to be promising biomaterials for tissue regeneration. During such conditions, it appears that the cells do not affect the gelation process (Shanshan et al. 2010). This indicating that host-guest complexation is robust enough for cell-related applications.

Polymer Inclusion Complexes (PIC)

In the late 1940s, Fuoss and Sadek published a turbidimetric study on polyion condensation (Dautzenberg and Rother 2004). With more sophisticated instrumentation, the process of particle assembly has been better understood for the past few decades. According to recent research, particles are formed when nonstoichiometric mixtures of oppositely charged polyions combine, creating a neutral core enclosed in a shell of excess polyion chains (Buchhammer et al. 2003). Researchers have established that mixtures of oppositely charged polyions in solution cause the formation of PIC particles that are reinforced by a charged corona, which repels neighboring particles. The hydrophobic attraction between neutral coacervates in stoichiometric polyions occurs during flocculation rather than equilibrium, and can lead to unstable shell-deficient PIC particles (Dautzenberg and Jaeger 2002).

Discrete polydisperse particles are typically composed of diameters between ten nanometers (nm) and one micrometer (mm). Indirect methods, such as static and dynamic light scattering, are typically used to determine particle size, which preserves the integrity of nanoparticles in solution. In addition to light scattering, imaging techniques are often used as a support technique; however, particles are often affected by various processes during the preparation and analysis of the samples (especially in the case of drying and staining) (Patterson et al. 2014). It has been demonstrated that the increase in entropy subsequent to counterion release from polyelectrolyte is a result of electrostatic attraction between polyions charged oppositely (Ueno et al. 2012). However, it has been demonstrated that the increase in entropy caused by counterions released from polyelectrolytes explains the overall behavior of PIC particles (Bucur et al. 2006).

Nanoparticles and free polyions in solution coexist in equilibrium during the self-assembly of PIC particles (Zintchenko et al. 2003). PIC particles are inherently unstable due to their dynamic nature, making them vulnerable to corrosion and electrostatic shielding with addition of other charged species (Sui et al. 2003). Therefore, the stability of PIC particles in biological environments may be seriously compromised, as they may be exposed to high concentrations of electrolytes and proteins with charges (Dash et al. 1999). By using cross-linking of the polyelectrolyte components, PIC particles can be easily stabilized, which would drastically

reduce the exchange with free polymers in solution. Conversely, damaging PIC particles with physiological electrolytes improves their permeability, allowing them to release preloaded charges (Cai et al. 2012).

A final point to note is that only certain polyelectrolytes can form stable PIC particles if they are mixed correctly under suitable conditions. It is important to optimize several factors during the formulation of these nanomaterials, including chemical composition, charge density, and charge concentration of the polyelectrolytes, pH, ionic strength, and relative ratios of both polyelectrolytes. It is essential to optimize all of these parameters at once since they are interdependent (Cheow and Hadinoto 2012). In certain conditions, coacervates separate from solution and coalesce into dense materials, which also show great promise for biomedical applications, such as synthetic cartilages and enzymatically active biocomposites (Fu and Schlenoff 2016).

Nanofillers

Polymer matrix nanofillers fill specific volumes, alter conformational states, and immobilize nearby molecular groups. In order to choose the right nanofiller, it is necessary to consider its physical and dimensional properties. In contrast to earlier composites, carbon nanotubes (CNTs) combine superior mechanical, thermal, electrical, and thermal properties (Yuan et al. 2017; Jeevanandam et al. 2018; Al Sheheri et al. 2019). An important aspect of characterizing nanofiller's application in polymer composites is their size (Lagarón 2011). The 3D, 2D, and 1D nanofillers fall under this category.

One-Dimensional Nanofillers

A one-dimensional (1D) nanofiller is defined as a filler with one dimension smaller than 100 nm (Lagarón 2011). In the form of individual sheets or hundreds and thousands of nanometer long sheets of material, they can be between one and a few nanometers thick. A common example of a 1D nanofiller is montmorillonite clay and nanographene platelets. Furthermore, nanostructures are also known as nanoprisms, nanosheets, multibranched structures, nanoplates, and nanowalls (Mittal 2015), and other shapes (Akpan et al. 2019). Layered silicates, MMT, graphite nanoplatelets (GNP), ZnO nanoplatelets, ZnO nanodiscs, carbon nanowalls, amphiphilic graphene plates, and ZnO nanosheets are noteworthy examples (Mittal 2015). The shape-dependent properties of most 1D nanofillers make them useful in the fabrication of nanodevice components. Microelectronics, biosensors, and sensors are among the applications that they are used for. These properties are due to their excellent electrical, magnetic, and optical characteristics (Akpan et al. 2019). Electrical and thermal applications are beneficial to 1D nanofillers due to their dimensionality.

Two-Dimensional Nanofillers

Two-dimensional (2D) nanofillers have less than 100 nanometers in their dimensions (Lagarón 2011). Tubes, fibers, and filaments are usually the most common shapes. The

most common 2D nanofillers include 2D graphene, black phosphorus, clay nanotubes, carbon nanotubes (CNTs), cellulose whiskers, boron oxide tubes, boron carbon oxide tubes, and gold or silver nanotubes. Polymer nanocomposites can be manufactured using 2D fillers. There are several others, including molybdenum disulfide (MoS₂), hexagonal boron nitride (h-BN) (Ribeiro et al. 2017), graphene, MoS₂, and graphene oxide. There are many applications for 2D nanofillers, including photocatalysis, nanocontainers, nanoreactors, energy, sensors, catalysis, electronics, and optoelectronics. In comparison with 3D and 1D nanofillers, they are found to confer superior flame-retardant properties. As well as providing more reinforcement, they are also shown to be less expensive than 3D fillers (Araby et al. 2021).

Three-Dimensional Nanofillers

An example would be nanoparticles with three dimensions that are relatively equiaxed in the nanoscale (nanometer). Nanoparticles are usually isodimensional or zero-dimensional, and are usually in spherical and cube shapes. A 3D nanofiller is also known as a nanosphere, nanogranule, or nanocrystal. The materials used to form 3D nanofillers include semiconductor nanoclusters, carbon black, silicon carbide, nanosilica, nanoalumina, nanotitanium oxide, polyhedral oligomeric silsesquioxane (POSS), nanomagnesium hydroxide, silica, and quantum dots (QDs) (Lagarón 2011). Polymer nanocomposites are formulated with 3D nanofillers because of their inherent properties. Nanoparticles (e.g., Fe₃O₄, ZnO, TiO₂, Ag, and SiO₂) exhibit high stability, hydrophilicity, and ultraviolet (UV) resistance along with excellent transparency to visible light, nontoxicity, and high photocatalytic activity. They are useful in applications like coatings, separation and purification, and biomedicine when coupled with polymers that have suitable properties (Mittal 2015).

Amphiphiles

An amphiphile is a chemical molecule that exhibits both hydrophilic (attracted to both water and fat) and lipophilic (attracted to fat) qualities. Amphiphile comes from the Greek words: *αμφίς*, *amphis*: both and *φιλία*, *philia*: love, friendship. Such substances include amphiphilic or amphipathic compounds. Lipoproteins, detergents, and soaps are examples of amphiphilic substances. Cell membranes are highly structured by phospholipid amphiphiles (Schubert et al. 1995). Researchers in chemistry and biochemistry consider amphiphiles central to several areas of study, including polymorphism of lipids. The chemical compound bolaamphiphilic contains hydrophilic groups at both ends of the molecule. These micelles are prolate. Often, amphiphilic species have a majority of hydrophilic parts, a majority of lipophilic parts, or a mixture of the two (Phillips 2015). An example would be proteins or block copolymers. Amphiphilic compounds have hydrophilic polar functional groups (representing either ions or anions). Amphiphilic compounds are also capable of dissolving in water and, to a lesser extent, in nonpolar organic solvents due to the existence of both lipophilic and hydrophilic parts. A compound that is amphiphilic will partition aqueous and organic solvents when it is placed in an

immiscible biphasic system. In particular, the extent of partitioning (Schubert et al. 1995) depends on the length of the hydrophobic and hydrophilic portions.

Biological Role

Membrane components are phospholipids, an amphiphilic class of molecules. Molecular membranes are formed as a result of their amphiphilic nature. Using two layers of lipids to form a sheet, they mutually organize themselves into lipid bilayers. Lipophilic chains form a layer by being positioned on the same side. The layers are then stacked so their polar groups are outside and the lipophilic chains touch on the inside. There is thus an internal nonpolar region to the bilayer sheet, which is patched between the two polar sheets (Schubert et al. 1995).

Biological membranes are largely composed of phospholipids, but also contain another class of fatty acids, cholesterol, and glycolipids, that confer different physical and biological properties to them (Watson 2015).

Many of these derivatives interact with biological membranes, making the hydrophobic part of them adhere to lipid membranes while exposing the hydrophilic part to water, altering the physical behavior of the compound (Peetla et al. 2009).

Antiparallel A β sheets form toxic oxidative A β fibrils, formed by bilayers of A β sheets which are strong amphiphilic. As the name implies, A β proteins are a combination of double β sandwiches consisting of 13-mer modules. In addition to describing the small fragments of A β (with 40 and 42 amino acids), hydropathic waves can also present a better description of plaques (Schubert et al. 1995).

AMPs with anti-gram-negative bacteria activities are another class of amphiphilic molecules. A study revealed that amphipathicity is the most useful measure for determining which AMPs possess these abilities. Antibacterial and antifungal dual-activity AMPs have a better chance of having high amphipathicity (Ramazi et al. 2022).

General Applications of Polysaccharide-Based Gels

Utilization of Agarose Gel

Agarose is made up of repeating agarobiose (L- and D-galactose) subunits and is found in the seaweed species like *Gelidium* and *Gracilaria*. A noncovalent network of agarose polymers controls the molecular sieving capabilities of gel during gelation by controlling the pore size (Lee et al. 2012). It is used in the separation of DNA fragments called agarose gel electrophoresis (AGE) (Lee et al. 2012). In electro-membrane extraction, an agarose gel can be used as a supported liquid membrane (SLM) between the acceptor and donor phases (Tabani et al. 2017). It is also used in the liquid phase microextraction for high extraction efficiency (Prosen 2014).

Alginate's Uses

Brown algae (*Phaeophyceae*) such as *Laminaria hyperborea*, *Laminaria digitata*, *Laminaria japonica*, *Ascophyllum nodosum*, and *Macrocystis pyrifera* are the main

sources of alginate. Sodium alginate has been widely used in biomaterial applications because of its biocompatibility and hydrophilic nature (Sahoo and Biswal 2021). They are used in the delivery of low-molecular-weight drugs to the target site. The alginate beads are used for the formation of primary and secondary bonding with the drugs which can be used for control drug release kinetics (Li and Mooney 2016). The benefits of alginate gel for bone and cartilage regeneration include their minimally invasive surgical techniques, ability to fix irregularly shaped defects, and ability to release tissue-inducing factors such as BMP and TGF- β (Lee et al. 2012). They are used as the physical barrier in the reduction of reflux episodes and in the treatment of heartburn and esophagitis exist (Szekalska et al. 2016). It can also be used in the treatment of obesity as it dissolves in stomach and stretches into a soft, gel-like substance that remains stable in the gastric acid environment and maintains satiety for 6–8 h, after which it dissolves in the small intestine due to the neutral or alkaline environment by formulating with standalone or various foods (Sarkar et al. 2020). Intake of alginate at higher dosage decreases the hunger and food uptake by increasing viscosity and tendency of feeling full due to the formation of alginate gel in the contents of intestine (Maljaars et al. 2008). In food industry, alginate beads are used in encapsulation of probiotics in order to increase the cell viability and storage stability (Gheorghita Puscaselu et al. 2020). It is also used in the reduction of ice crystal formation during freezing of ice creams, in order to prepare smooth product. Alginates were used in the preparation of dessert and jellies which do not melt but only at body temperatures (Qin et al. 2018). Frozen fishes are preserved using the utilization of calcium alginate films and coatings. Due to the property of forming viscous solution by dissolving in water, alginates are used as thickening, stabilizing, emulsifying, and gelling agent. These are also used in the immobilization of enzymes in fruit juices, syrups, oils, etc. (Brownlee et al. 2009). In cosmetic industries, it is used in the formation of skin-protective creams in order to prevent industrial dermatitis and in shaving preparation as stabilizer for easier spreading, rinsing, and lowering the solid content of the cream (lesser, 1950) (Fig. 3).

Employment of Collagen and Fibrin

Collagen's biological function makes it very promising for use in the pharmaceutical, therapeutic, and aesthetic sectors (Avila Rodríguez et al. 2017). It is used in the cosmetics formulation as it has property of film formation, regeneration, and skin moisturization (Sionkowska et al. 2020). Collagen is used in the production of beauty masks (hydrogel) which helps in the restoration of skin's elasticity and helps in the promotion of antiaging performance (Sionkowska et al. 2020). The topical administration of fish collagen contained negatively surface-modified nanoliposomes promotes antiaging performance by increasing the Type I procollagen protein expression (Subhan et al. 2020). Collagens are used as edible films and coatings for preventing the migration of oxygen (O_2), moisture, and solute, for providing the structural integrity and permeability of vapor (Hashim et al. 2015).

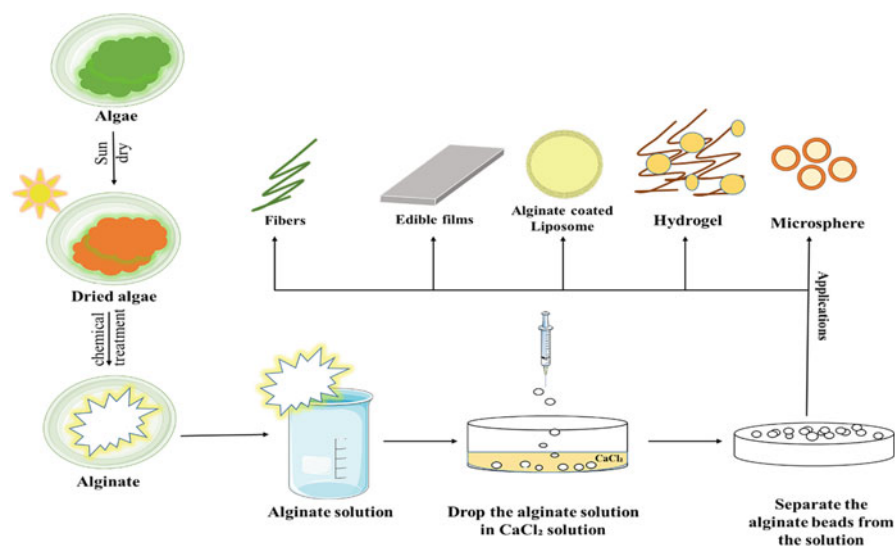


Fig. 3 Uses of alginate extracted from algae

It is also used as food additives such as colorants, antioxidants, preservatives, emulsifiers, and thickeners (Hashim et al. 2015). Fibrin, an extracellular matrix, is used in the cell-matrix interactions and in tissue engineering such as cardiac micro-environment designing, tissue transplantation, and bone regeneration (Litvinov and Weisel 2016). It also plays a major role in drug delivery by loading of drug through impregnation (Ahmad et al. 2015). Fibrin is used as a coating agent in bone tissue engineering for cell growth factor attachment and scaffold distribution (Noori et al. 2017).

The Role Played by Gelatin

Gelatin is used in the food industry for manufacturing desserts, for texture foaming clarity and as a stabilizing agent (Alipal et al. 2021). It is also broadly used in the field of cosmetics as gelling agent in shampoos, hair sprays, sunscreens, body lotions, and facial creams (Kamatchi et al. 2016). In pharmaceutical industry, gelatin is used as topical drug delivery system such as skin burns, capsule coats, binding tablets, sponges for treating wounds, and for vitamin formulations (Petros et al. 2020). Gelatin is widely used in the production of capsule which is mostly preferred over tablet in medicine administration (Sebastian 2014). In food industry, 1% gelatin is used as coating in lozenges, candies, and wafers and used as clarifying agents in juice industry to remove the suspended particles in juice (Petros et al. 2020). Two-dimensional gelatin substrates were used in the cell and tissue cultures, especially the fibronectin-

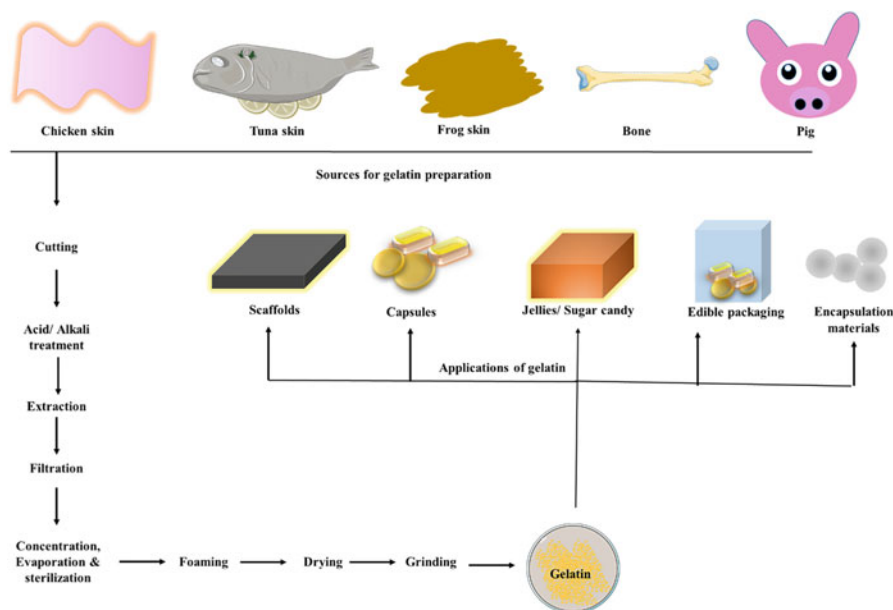


Fig. 4 Preparation of gelatin from various animal sources and its applications

producing cells as it binds with dishes coated with gelatin more efficiently (Bello et al. 2020). In earlier studies, it has been reported that channel catfish skin gelatin–incorporated ice cream had enhanced mouthfeel by stabilizing high emulsion foaming (Ranasinghe et al. 2022). Channel catfish gelatin is also used in the clarification of beer by protein flocculation (Duan et al. 2018) (Fig. 4).

Hyaluronic Acid

Hyaluronic acid is used in the treatment of cancers such as prostate and carcinomas as tumor marker and in monitoring the mucoadhesive progression (Lokeshwar et al. 2014). It is used in the targeted drug delivery due to the binding of hyaluronic acid and its derivative to specific cell surface receptors mainly present in liver, kidney, blood vessels, and body fluids (Gupta et al. 2019). Due to degradation of the components like hyaluronic acid, extracellular matrix is the cause of skin aging which leads to decrease in skin tone, wrinkles, and sagging (Papakonstantinou et al. 2012). Hyaluronic acid is used as skin patch in order to overcome the above issues (Juncan et al. 2021). It is used in osteoarthritis for the restoration of synovial fluid viscoelasticity (Saranraj and Naidu 2013). It plays important role in the cellular and matrix meditation, hence used in wounds healing such as abrasions, donarsites, postoperative incisions, and ulcer (Saranraj and Naidu 2013).

Pros and Cons of Using Biopolymer Gels

Biopolymer-Based Gels	Pros	Cons
Chitosan	It is used as antimicrobial and antioxidant (Schueller and Romanowski 1999)	Chitosan intrinsic properties are affected by cross-linking of chitosan gel (Ishihara et al. 2001)
	It has excellent adhesion with cells	It has high viscosity to the solution
	It is from renewable resources	Transformation into nanofiber is difficult It is insoluble in many solvents
Collagen	It enhances the skin hydration and skin elasticity. It reduces wrinkles (Goodarzi et al. 2019)	It results in cutaneous necrosis and inflammatory reactions on a local level (Seal et al. 2001)
Alginate	It smoothes out fine creases and improves skin elasticity and strength. It gives the appearance of younger skin (Kozłowska et al. 2018)	The resulting gel may have an unpleasant odor (Takahashi et al. 1990)
Xanthan gum	It withstands a wide variety of temperatures and pH levels, has skin-conditioning effects, and is nontoxic (Lopes et al. 2015)	It irritates the skin (Lopes et al. 2015)
Kappa-carrageenan	It attracts water to the skin and hair. It is utilized as a hair conditioning agent. It has a moisturizing effect (Noor 2018)	It might cause irritation (Noor 2018)
Pectin	It strengthens skin (Ammala 2013)	Pectin from a few sources may have low gelling ability on occasion (Ammala 2013)
Guar gum	It enhances hair and skin. It keeps water from evaporating. It extends the life of the product (Singh et al. 2014)	It is hypersensitive towards allergens (Singh et al. 2014)

Conclusion and Future Perspectives

Cellulose, polysaccharide, starch, and chitin are used to create biopolymer-based gels, which are renewable, biocompatible, and biodegradable. However, recent studies are primarily focused on potential applications of biopolymer gel based water purification and pharmaceutical and medical systems, with less attention paid to other areas such as electrical sector. The development of new biopolymer-based gels using natural or chemically modified biopolymers have gained substantial interest as a result of recent improvements in our understanding of how to isolate, describe, and use different types of biopolymers. The development of scattering and microscopical approaches, the incorporation of structural and rheological techniques

in the study of gelation kinetics, the use of mean field theories, and the pursuit of new simulation techniques are some of the major accomplishments in the field of biopolymer gels.

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D. H. Nagaraju (✉)

Department of Chemistry, School of Applied Sciences, Reva University, Bangalore, India

e-mail: dhnagu@gmail.com

S. Budagumpi · Z. Yhobu

Centre for Nano and Material Sciences, Jain University, Bangalore, India

e-mail: b.srinivasa@jainuniversity.ac.in; y.zhoveta@jainuniversity.ac.in

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Abstract

The current era is challenged with depleting oil reserves and ever-increasing pollution from petroleum-based fuel pollutants. Faced with this challenge, there is a growing need for the development of renewable and sustainable materials that can potentially replace the conventional materials. The present governments and world organizations have instilled a major focus in making the world cleaner, pollution free, and reducing the usage of fossil fuel. This focus has driven industrial and governmental entities to stress on the research, development and fabrication of finished materials from sustainable, renewable, and recyclable biopolymer-based composites. As alternates to conventional materials, biopolymer-based composites are expected to attain the same or even better performance. Biopolymers possess the inherent property of being functionalized during their preparation enabling better compatibility and efficient models for preparing composites. This book chapter gives an overview of biopolymer-based composites; their physical, chemical, and material characteristics, the various synthesis approaches and their applications in automobile industries, medical field, biosensing, food packaging, water treatment, and energy storage devices.

Keywords

Biopolymer · Composites · Sustainable · Fibers · Polymer

Introduction

The demand for sustainable materials with prominent properties increasing which could mitigate the environmental issues posed by fossil fuels. To fulfil these requirements, materials that are stand alone have few intrinsic limitations to be utilized in high performance applications. However, interesting, fascinating, and high-performance property materials can be achieved by the development of composite materials. A composite is developed from two or more materials with distinct varying properties, the unification of these materials results in the formation of materials possessing the properties of its precursors. Composite materials result in enhanced and multifunctional superior materials. Biopolymers being renewable and biodegradable have garnered immense interest and are predicted to replace the largely popular petroleum-based polymers. Biopolymer-based composites are

prepared with the aim of achieving good mechanical behavior, durability, properties of both natural fiber and biopolymer (Ramamoorthy et al. 2015). In this regard, biopolymer composites are biopolymers that are reinforced by natural fibers, polymers, fillers, etc. The reinforcing of biopolymers affects their crystallinity, thermal properties, morphology, production cost, and durability. Biopolymer composites have also gained immense popularity in light of the commonly used polymers ill-effects on the ecosystem due to their non-biodegradability. The recycling of these commonly used polymers by the popular recycling process, i.e., thermal recycling more commonly called incineration ends up affecting the overall quality of stability. Biopolymer-based composites on the other hand can be recycled by similar recycling process for several cycles with minimal change in its properties (Ngaowthong et al. 2019). These qualities of biopolymer-based composites show great promise for scaling and widespread usage as end products/commercial products. Biopolymer-based composites have already found their place in various applications like food industry, biomedical applications, packaging, water purification, and structural constructions (Mohan et al. 2016).

Biopolymer-based composites that are naturally available are classified into wood-based fibers and non-wood-based fibers. Biopolymer-based composites of varying type is made up largely of lignin and cellulose; the difference in percentage of these two components play a huge role in the physical property and performance like tensility, crystallinity, and strength.

This chapter discusses the preparation methods of biopolymer-based composites, their properties, and their application in various fields.

Preparation of Biopolymer-Based Composites

Biopolymer composites can be synthesized from various methods from wide variety of biopolymers and natural fibers. The combination of different biopolymers with different natural fibers can result in various innumerable outcomes. Many approaches can be taken while developing a biopolymer composite depending on utility, starting materials employed, time, and resource availability. There are advantages because the biopolymer-based composites can overcome individual limitations of the starting materials used. Some of the more popular preparation techniques have been described in this section.

Graft Polymerization

Graft polymerization is the process in which a polymer chain serves as the backbone upon which different monomers get bonded to it and polymerase as side chains. Graft polymer involves two types of polymers where the polymer that make up the main chain and the side polymer chain are distinct and different. The monomers that made up the side chains can be of different types hence making this technique an attractive technique for grafting different functional groups to the main polymer

chain. There are two grafting approaches, i.e., “grafting to” and “grafting from,” the “grafting to” involves the monomers reacting with the polymer backbone, the “grafting from” involves polymer backbone being functionalized to bind with monomers. Grafting process can be initiated by chemical treatment, ionizing radiation, enzymatic grafting, plasma-induced, photochemical treatment, and radiation (Sherazi 2016). Grafting polymerization is one of the most popular methods of functionalizing cellulose that is carried out mostly by free radical polymerization, living radical polymerization and ionic and ring opening polymerization (Udayakumar et al. 2021).

In-situ Polymerization

This technique involves the formation of a reinforced phase, it involves the dispersion of the fiber along with the monomer in solution followed thorough mixing and homogenization of the precursor solution. An initiator or catalyst is added to the system to assist in the formation of free radicals by breaking bonds as part of the chain reaction; this system is simultaneously treated by light or heat for curing. In case of thermoplastics, the initiator and monomer are directly added to mold resulting in the formation of matrix with the mold in a single step. This process is unique because the molecular weight of the composite can be manipulated by manipulation of the amount of initiator in the monomer solution. This technique can be used for making complicated shapes and is a useful fabrication tool for making implant materials (Sadasivuni et al. 2020).

Infiltration Method

This method consists of four steps (i) Pre-form fabrication, (ii) Interphase deposition, (iii) Infiltration, and (iv) Thermal processing. In pre-form fabrication, the shape of the desired product is molded to form the fiber reinforcing phase, this is followed by interphase deposition where the fibers are coated by means of interphases to form a fibrous pre-form. The reinforced fibrous perform is filled by infiltration with ceramic matrix that contains fillers and particulates of interest, this is followed by thermal treating of the ceramic matrix with reinforced fiber to yield the desired product of desired shape. There are various infiltration methods for fabricating biopolymer-based composites like polymer infiltration and pyrolysis, slurry infiltration, sol-gel infiltration, chemical vapor infiltration, direct melt oxidation, liquid silicon infiltration, etc. (Sadasivuni et al. 2020).

Phase Separation

Phase separation, also referred to as phase inversion is a versatile technique for preparing biopolymer composites. It is centered on the precipitation or solidification

of the phase with polymer from a system that was originally homogeneous. The homogeneous solution is prepared by dissolving the polymer in an appropriate solvent. The thermodynamics behind this process is the miscibility gap that exists between the polymer and the solvent. Conditions are applied such that polymer starts to precipitate due to the dissolving ability of solvent reducing with respect to the polymer. There are various ways to carry out phase separation, among them thermally induced phase separation (TIPS) is the most widely accepted and known method. TIPS employs an apt solvent that does not dissolve the polymer at normal room temperature but is able to dissolve at lower temperatures to obtain a homogeneous solution. The homogeneous solution is casted or extruded with different architecture with lower temperature to induce phase separation. The solvent is then exchanged with volatile organic solvent so that the solvent can be dried and removed easily. TIPS is aided with binary phase diagram to understand phenomenon of the phases between the polymer and solvent that are stable, unstable and metastable. TIPS is commonly used for the preparation of biopolymer composite membranes that are porous, lightweight and flexible (Figoli 2020).

Intercalation

This method involves the insertion of polymers into layered substrates like silicates and is particularly useful for preparing organic-inorganic hybrids. There are three types of intercalations depending on the processing technique and the starting materials used: *in situ* polymerization intercalation, melt intercalation and polymer solution intercalation. Among the three processes, polymer solution, intercalation, and melt intercalation are the more commonly used. Polymer solution intercalation involves dissolving the polymer/monomer with appropriate solvent and swelling of the layered substrate. The layered substrate is swollen using a solvent which is mixed with polymer and the polymer intercalates and ejects the solvent in the layered substrate to produce intercalated structure. This is useful when employing water soluble polymers, however this system is only possible for a few selected polymers. However, the excessive use of solvents that are harmful and expensive makes this method difficult to scale. The limitations of polymer solution intercalation have enabled melt intercalation, this method is eco-friendly since harmful organic solvents are not needed and the scaling and processing process is easier. This method can use polymers which are not suitable in the other two methods (Shen et al. 2002).

This intercalation technique is especially helpful for the preparing metal-based composites by the mixing of metal precursors and biopolymer; however, this technique is limited to very few biopolymers like poly-lactic acid, cellulose, lignin and is not suitable for polymers like chitosan and poly(vinyl alcohol).

Solvent Casting

Solvent casting, also referred to as wet processing or solution casting is a popular method that is simple and does not require specialized instruments. Solvent casting

mixes the polymer matrix and filler by continually stirring and agitating, the mixture is used for casting followed by eventual solvent evaporation and drying. The first step is dissolving the polymer to make a polymer solution with an appropriate solvent, most commonly used solvents are water and alcohols. The polymer solution is often heated or the pH is changed to enable optimum conditions for the formation of the film. The polymer solution is employed for casting into a mold or a film by casting on a flat surface, this is followed by the removal of the solvent. The film maybe subjected to heat treatment to remove stress. There are several criteria's that should be considered in this process: the polymer should be soluble in the solvent; minimal solid content should be present; viscosity, the film should be as homogeneous as possible. Factors like the choice of solvent and the molecular weight of polymer play an important role in the quality of the film formed. There are also several limitations in employing this process such as the requirement of large amounts of solvent which hinders its scaling for industrial applications, the possibility of toxic solvent being retained, time consuming, the possibility of proteins and drugs denaturation due to the solvents employed, solvent recovery is difficult. Despite these limitations, it is still considered one of the best choices for fabrication quality high films and better performance which is difficult with other methods. This method is widely used for preparing coating for biological applications and packaging applications (Kong et al. 2015).

Layer-by-Layer Deposition (LBL)

This method is another popular and sustainable route for developing thin film biopolymer-based composites. This technique involves the deposition and stacking of thin films of different materials alternately with the different layers of films complementary to each other. The LBL technique is one of the most common methods for developing bilayer material. It is also complimented by other techniques like spray coating, dip coating, spin coating, etc. It has been employed as a technique for depositing materials like nanoparticles (NP), carbon nanotubes (CNT), biological molecules, electrolytes, etc. (Sadasivuni et al. 2020). Another advantage, it offers precise control of the film. The bonding and interaction involved in LBL composites are covalent bonding, hydrogen bonding, electrostatic forces, and weak intermolecular interactions. Other advantages of using this technique is that it is highly facile and feasible (Tang et al. 2012).

Coagulation Spinning

This technique is more commonly employed for fabricating polymer yarns, fibers and polymer- nanotubes composites. There are two types of spinning, i.e., wet and dry spinning. In dry spinning, the solvent present in the fiber is evaporated by heating whereas in wet spinning, the solvent is removed by coagulating with another solvent. This technique involves injection of surfactant-stabilized filler dispersion into polymer solution like PVA in a rotating bath. The NTs and polymers are made to

flow in the same direction by injection, the polymer molecules ensue in replacing the surfactant thereby destabilizing the nanotube (NT) dispersion. The replacing of surfactant and destabilizing of the NT dispersion makes it collapse to form a fiber, which is extracted and derived by coagulation from coagulation bath (Sadasivuni et al. 2020). This method is a popular method for fabricating CNTs by either wet spinning or dry spinning. There are two types of spinning, i.e., wet and dry spinning. In dry spinning, the solvent present in the fiber is evaporated by heating. In some instances, the mechanical property dry spinning is superior to wet spinning; however, it has several limitations like its scalability and less production quantity which affects the overall production quality. Wet spinning on the other hand is scalable, simple, easily produced and requires mild temperature conditions (Berber and Hafez 2016).

Electrospinning

This is one of the most preferred techniques for synthesizing biopolymer composites from their respective melts and solutions. It employs a high electrical field where a polymer is charged and accelerated to form ultrafine NFs ranging from 10 nm to 1 μ m. A conventional electrospinning is made up of a syringe that is filled with polymer either in solution or melt form, the tip of the metallic syringe is connected to either the positive or negative terminal of the power supply with the other terminal usually grounded. The syringe is connected to a pump which constantly pumps out polymer producing nanofibers (NF) that is collected at the other end of the terminal. There are several parameters that regulate the formation of polymer NF like the solvent employed, the concentration of solvent, concentration of polymer, applied voltage, viscosity of solution, surface tension, distance between tip and collector, flow rate, collector type, temperature of the system, etc. Composites produced from electrospinning are used in applications ranging from sensors, filters, scaffolds for biomedical purposes to fuel cell membranes, organic electronic, energy storage devices, etc.

Electropolymerization

This method is utilized for depositing polymers onto a conductive surface or substrate and is particularly used for coating small areas. Electropolymerization works on the principle of applied current or potential resulting in the oxidation of monomers and depositing the polymer film onto the surface. The electrochemical polymerization is usually carried out in an electrolytic cell that contains the conductive substrate on which the coating or film formation will be done and is called the working electrode. The electrolytic cell also contains inert electrodes, i.e., reference electrode and counter electrode against which the potential and current applied in the cell is measured. The cell is filled with the electrolyte which contains dissolved monomer, metal salts, redox active molecules, etc. The electrolyte may also contain

a supporting electrolyte to render the electrolyte more conductive so as to enhance the polymerization rate. Electropolymerization can be divided into potentiodynamic, potentiostatic and galvanostatic methods for polymerization. Potentiodynamic technique involves a sweeping of potential between two points in which the monomer oxidizes. The polymer film grows with increasing sweeps and is accompanied by adsorption and desorption of the electrolyte to stabilize the polymer film. Galvanostatic electropolymerization works on the principle of applying a fixed current at which the monomer oxidizes at a constant rate. Potentiostatic electropolymerization is similar to galvanostatic, however in this technique a constant potential is applied. Electropolymerization is a technique that is limited to monomers that are electroactive and thus this limits its application. This technique has been extensively used for polymerizing redox active small biomolecules that contain quinones and functional groups. An advantage of this technique is the ability to simultaneously deposit other electroactive materials like metals, conducting polymers, while polymerizing the redox active biomolecules/ bio-monomers. This method is advantageous in that high-quality polymer chain and uniform polymer deposition is achieved. This technique is particularly useful for the construction of energy storage devices. The limitations of this technique is the scalability issues and the requirement for the monomer to dissolve in the electrolyte (de Leon and Advincula 2015; Nagaraju et al. 2014).

3D Printing

3D printing is grabbing lots of attention in the fabrication of biopolymer-based composites and scaffolds particular for biomedical applications like tissue engineering and regenerative medicines. 3D printing has advantages over other fabrication methods due to its more precise control over the geometrical parameters like porosity, pore size, and pore interconnection size. 3D printing is a method of fabricating 3D materials and objects with shapes that are designed digitally. It is an additive manufacturing technology that is used for fabricating a target shade by addition of layers successively. It is a technique that is used for both designing the prototypes and manufacturing the products which are used in a wide array of applications. In 3D printing, the first step is designing the model by means of computer-aided designs (CAD) and other modeling softwares that directs the machine in the successive printing process. The second step is the printing where the machine interprets and analyses the data and model that has been designed and starts laying/printing successive layers with the material which can be in liquid, powder, or a combination of both. In most instances, the 3D printing employs several materials for the printing process and supports are also employed sometimes for the printing which are removed upon the completion of the printing. Depending on the application of the printed object, post processing may also be carried out. The additive process can vary from materials and applications, some of the more popular processes that uses biopolymers are fused deposition modeling, direct metal laser sintering, stereolithography, and selective laser sintering. Some of the most commonly used biopolymers used in 3D printing are poly(lactic acid) (PLA), poly

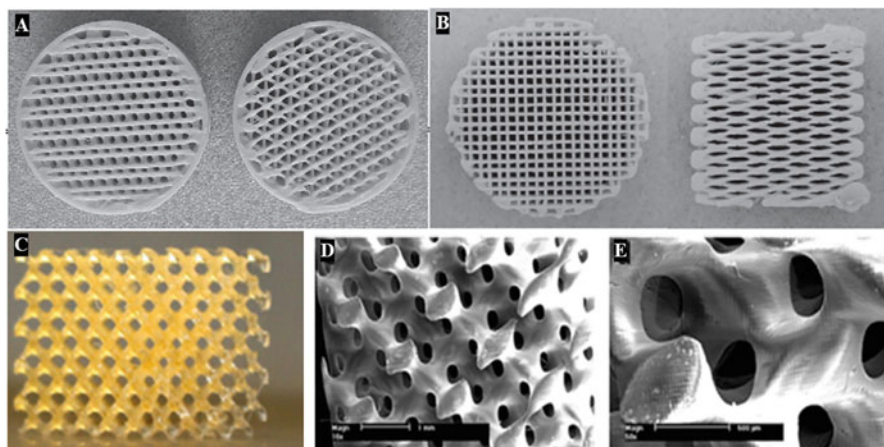


Fig. 1 (A and B) 3D printed composite scaffolds of constructed with Polypropylene (PP) and tricalcium phosphate exhibiting different internal architecture using fused deposition modeling. Reprinted with permission from (Kalita et al. 2003). Copyright Elsevier 2003. (C, D, and E) 3D printed scaffolds of poly(ϵ -caprolactone) constructed using stereolithography and its morphology studied with SEM. Reprinted with permission from (Elomaa et al. 2011). Copyright Elsevier 2011

(glycolic acid) (PGA), poly(lactic-*co*-glycolic acid), etc. (Li et al. 2014). Fig. 1 show different types of architectures that can be constructed with different printing process (Elomaa et al. 2011; Kalita et al. 2003).

Other Methods

Extrusion is a method used for producing composites of fixed cross-sectional area by pushing the material through a die of the required cross-sectional area. Common examples of materials produced through this process are polymers, ceramics, metals, modelling clay, etc.

Pultrusion is a method similar to extrusion used for producing fiber-reinforced materials, compared to extrusion, it pulls the material instead of pushing like in extrusion.

Filament winding involves the winding fibers on a rotating mandrel, the fibers are then mounted and processed to form fibers that are wrap in different configuration like helical, hoop winding or arranged longitudinal (Verma and Fortunati 2019).

Properties of Biopolymer-Based Composites

The properties of biopolymer composites are attributes of the intrinsic property of the starting materials, processing conditions and the state of the final product. The intrinsic property are characteristics like crystallinity, density, chemical structure and

composition, solubility, transparency, gas properties, etc. The processing characteristics are the dependent of the melt temperature, viscosity, index, strength, etc. The combination of the both the intrinsic and processing properties influences the product properties like its heat resistance, water resistance, aesthetic properties, electrical properties, mechanical properties, physical properties, biocompatibility, etc. Some of the important properties that are considered in the construction of the biopolymer-based composites will be described in this section.

Mechanical Properties

The mechanical integrity of the biopolymer-based composite is highly dependent on the application and the purpose for which it was constructed. The biopolymer-based composite is scrutinized for its mechanical properties like tensile test, compressive, creep behavior, flexural, flexibility, impact, hardness, shear, etc. These mechanical properties are governed by factors like the polymer type, molecular weight, crystallinity, chemical composition, type of linkage, orientation, morphology, and concentration. It has been shown in various studies that the tensile strength of the material can be enhanced by reducing the hydrophilicity. The tensile strength has also been found to be increased by treating the fiber involved. The orientation of fiber either in longitudinal or transversal direction also influenced the tensile strength which can be employed for designing composites. The mechanical properties can also be enhanced the addition of nanofibers, nanomaterials, nanoparticles into the polymer matrix (Ramamoorthy et al. [2019](#)).

Dynamic Mechanical Thermal Properties

This analysis is an extension of mechanical analysis and heat which investigates the composites' ability to deflect heat, it gives information of the composite with regard to the elastic property/storage modulus, viscosity/loss modulus, and material damping ($\tan \delta$) as a function of temperature. These properties are analyzed by thermal analysis techniques like thermogravimetric and differential scanning calorimetry. The dynamic property of the material is associated and affected by the polymer, plasticizer, and the reinforcement technique employed (Aaliya et al. [2021](#)).

Thermal Properties

Thermal property like the thermal stability and conductivity of a biopolymer-based composites in an important parameter in determining the scope of the composite that is fabricated. There are various factors that determine the thermal property of the composite like the crystallization temperature, melting temperature, glass transition temperature, thermal degradation rate, heat of fusion, degree of crystallinity, etc. These parameters can be determined by using thermal analytical techniques like

differential scanning calorimetry and thermal gravimetry analysis. The thermal stability of a biopolymer composite can be enhanced by the addition of other biopolymers, one such instance is the combination of lignin with other copolymers. Lignin, an aromatic molecule that has numerous phenolic hydroxyls in its structure, results in various intermolecular hydrogen bonding with polymers that contain electronegative groups. This bonding between the polymers largely increases the thermal stability of the composite (Sen et al. 2015).

Tribological Properties

Tribology is the study of friction, wear and lubrication between two interacting surfaces in natural and artificial environments. It is most commonly investigated by “Pinon disc” test which involves the sliding wear between two surfaces within a fixed contact area. The property is crucial in determining the biopolymer-based composites durability and performance for practical applications in various scenarios and physical conditions. It has been pointed out that the inclusion of natural fibers enhances the tribological properties of the composite (Vinod et al. 2020).

Flame Retardancy

Biopolymer composites are becoming more commonly used in construction materials, textiles, parts of machines, etc. In view of their increasing usage and abundance, the flammability and flame retardancy of the composites is an important parameter that should be checked before being issued and used commercially. Natural fibers in its nature are highly flammable and produce heat exothermically on burning and for this reason they are commonly used as fuel in fire making since the primitive ages. There are various factors that affect the flammability of biopolymer-based composites like the type of fiber and biopolymer, structure of composite, adhesion between the matrix and reinforcement. The chemical composition and structure of fibers differ from one to another and hence the flame retardancy also varies. Cellulose which constitutes majority of the chemical composition of natural fibers is highly flammable and the increase in its content increases the flammability. It decomposes and emits flammable volatiles, tar which leads to char formation and non-flammable gases. Lignin, on the other hand, enables the char formation and protects the biopolymer composites as an insulator. It may be noted that high crystallinity and low polymerization of a polymer increases the flame retardancy. Flame retardancy of biopolymer composites are tested by Bunsen burner test by either vertical or horizontal test or the combination of both the test. To improve the fire retardancy of biopolymer composites, there are various techniques like the addition and dispersion of fire retardant agents in the composite, the addition of fillers that are endothermic in nature and the employment of intumescent systems that are painted over the composites which on exposure to fire expand and suffocate the burning by limiting excess to oxygen (Faruk and Ain 2013). Despite the

advantages that comes with employing various flame retardant agents, there are also limitations that are imposed on the biopolymer composite wherein the addition of flame retardant agents like halloysite NT was found to inhibit the mechanical ability of the composites. Therefore, there is a need to fabricate and seek new routes where the addition and inclusion of flame retardant agents to biopolymer composite does not affect either its properties or its performance.

Optical Properties

The commercialization of biopolymer composites in various industries requires thorough determination of their stability and its behavior. Optical property and stability of the composites play a crucial role in their employment as viable materials for coating, plastics, transparent materials and glazing agents. Factors like refractive index, dielectric constant, light transmission and absorption, color, polarizability, transparency and absorption coefficients are major areas where the composites are tested and considered. The stability of the composites when exposed to UV radiation is an important factor when considering the scalability of a composite. Natural fibers which contain lignin on exposure to UV radiation degrades giving out yellow discoloration and exposes other constituents to UV light. Sunlight cleaves the covalent bonds that is present in majority of the biopolymers causing degradation mechanically, discoloration, physicality and roughens the composite. Biopolymers composites are largely used in food packaging, the food should be preserved and ensured protection from UV light. This task can be achieved by increasing the refractive index of the composite, increasing the opacity by increasing the crystallinity of the biopolymers. There are various routes to achieve this target some examples are the addition of thyme essential oil to reduce transparency in starch-chitosan composites, addition of nanoparticles of silver, titanium, and copper to increase the refractive index (Jafarzadeh and Jafari [2020](#)).

Moisture Absorption

The performance of biopolymer composites reacts negatively to moisture, composites when they come in contact with moisture tends to plasticize and lose its toughness. The degree of expansion of biopolymer and the natural fiber varies which causes compatibility issue between the two and damages the interface between the two. Natural fiber on absorbing moisture swells up and shrink affecting its physical characteristics like flexural and tension modulus negatively. The swelling of fibers decreases the dimensional stability and allows for moisture to come in contact with the fiber-matrix interface causing delamination. This results in the fiber-matrix debonding caused by the osmotic pressure developed due to the leaching of water-soluble substances. This problem can be solved by avoiding the usage of hydrophilic biopolymers like cellulose and starch and switching to hydrophobic

polymers like poly(hydroxybutyrate-cohydroxyvalerate) (PHBV) and poly-hydroxybutyrate (PHB) (Christian 2019).

Biodegradability

Biodegradability of a biopolymer composite is another important aspect of biopolymer composite that depicts the microbial growth on it. The study of how various biopolymer composite degrade should be observed carefully to ascertain their application. Composites degrade in various conditions, for example natural fiber-reinforced biopolymer composites like PLA, PBS (polybutylene succinate) and PHB degrade by means of enzymatic degradation and anaerobic degradation. The process of biodegradation has been studied extensively and one such study evidenced the composites degrade by microbes making holes and cracking it to reduce its mechanical property (Alvarez et al. 2006). The protection of the composites from biodegradation is another important task, this can be achieved by making the composites less hygroscopic and enzyme resistant, surface modification to reduce the moisture content to inhibit microbial growth, delaying microbial growth by using harsh chemicals against microbes. The fabrication of composites using materials like lignin and PLA has been found to make the composite more resistant to microbial attack. The monitoring of biodegradability of biopolymer composites or any other material is however can be very difficult to consider because there is no standard procedure or test to monitor biodegradability. This issue leads to difficulty in testing of composites and the eventual optimisation of the composites toward biodegradation. This issue ultimately must be compensated by employing eco-friendly raw materials when constructing composites by considering its proposed functionality and applications. The considerations should be encompassed on properties like thermal stability, moisture adsorption and mechanical properties of the raw materials. The matrix of the biopolymer and the fiber should also be considered to yield the optimum properties for the biopolymer composite (Aaliya et al. 2021).

Applications

Water Treatment

Increased demand for and better quality of water has invented various methods and techniques to recycle, reuse and purify water for human needs. Water contains impurities in the form of total dissolved salts, natural organic matter, and heavy metal ions; other factors that ensure the safeness of the water are the alkalinity, hardness, biochemical and chemical oxygen demand, pH, and color of water. The purification of water from these impurities as well as ensuring the quality of water can be achieved in different stages of water treatment like coagulation, flocculation, remediation using membranes, physicochemical treatment methods like aeration, agitation, chemical precipitation, oxidation, disinfection, enzymatic decomposition,

halogenation, flotation, adsorption, ion-exchange, electrolysis, photolysis, etc. Traditionally coagulation and flocculation were the major methods used for treatment of water, the advancement in membrane technology as more cost effective and eco-friendly has made the two methods obsolete. Recent advances in water treatment using membranes are shifting in the direction of reverse osmosis and electrodialysis. Among the various methods that are present, choosing the appropriate treatment method depending on the pollutant and quality of the water ensures for more efficient usage of resources.

Heavy metal ions are becoming more distributed and widespread in the environment and their toxicity has become a serious concern. The removal of these metal ions in waste water discharged from industries is extremely important. One of the most effective methods to remove heavy metal ions is by using materials that occur naturally like alginate and chitosan. Alginate is a polysaccharide biopolymer made up of D-mannurinic acid and L-guluronic acid that are linked by β -1,4 linkage. It is known to have high affinity toward divalent metal ions and on contact with the metal ions forms gels. Chitosan another biopolymer that is derived from chitin has also been found to have high affinity toward heavy metal ions, the amine groups that are present on chitosan undergo chelation with heavy metal ions and neutralize their toxicity. In a study, the combination of these two naturally occurring biopolymers for the construction of adsorbent was examined. The alginic acid and the chitosan were used for preparing gel beads by the cross-linking between the amino and carboxyl group of the chitosan and the alginic acid, respectively. The gel was further treated with CuCl_2 to form gel bead particles, the gel was found highly durable in acidic conditions and adsorbed heavy metal ions of Cu(II) , Co(II) , and Cd(II) reaching equilibrium within 10 mins at 25 °C (Gotoh et al. 2004).

The combination of these two biopolymers has also been used for the preparation of membranes for remediation of herbicides, herbicides at various concentration ranging from 200 Mm to 5 mM were adsorbed by the membrane made from alginate and chitosan. The herbicides diquat and difenzoquat were tested, and it was found that the bilayer membranes adsorbed around 95% of diquat and 12% of difenzoquat at 120 min. These results were attributed to the coulombic interactions between the carboxyl group of alginate in the bilayer membrane and the positive charge on the herbicide, other factors like dissociation constant, partition coefficients, and electrostatic charge also influenced the performance of the membrane (Agostini de Moraes et al. 2013).

Biopolymer composites can also be used as adsorbents for remediation of fluorosis caused by the presence of fluoride ions in drinking water. There are so many prevalent materials that are used as adsorbents for fluoride treatment. Biopolymer composites as materials have advantages over other materials in terms of its biodegradability, low cost, and biocompatibility. One such example is the treatment of fluoride ions by development of adsorbent from alumina and chitosan. Alumina as a material has high textural and physical properties, low cost, easy regeneration, and high affinity for fluoride ions. These features of alumina have made it a common filler for removal of fluoride. Much like alumina, chitosan has excellent biodegradability, biocompatibility, non-toxicity and adsorption properties as highlighted in this

section. The combination of these two materials resulted in excellent fluoride removal and is evidenced by the SEM micrographs in Fig. 2a, b, where the particle size of the composite increases on adsorption of fluoride, the Energy Dispersive X-Ray Analysis (EDAX) spectra also confirms this as shown in Fig. 2c, d. The IR spectra of the composites before and after fluoride adsorption is also shown in Fig. 2e (a and b) wherein the band at 1379 cm^{-1} is sharper, stronger, and shifts to 1385 cm^{-1} after fluoride adsorption. The removal of fluoride ion by the composite is attributed to the electrostatic adsorption wherein Al^{3+} attracts the fluoride ions that are negatively charged. Simultaneously, there is complexation of the positive surface to the fluoride ions with fluoride as chelating agent. The defluoridation capacity was found to be higher than most fluoride adsorbents reported (Viswanathan and Meenakshi 2010).

Another major water pollution issue is the presence of dyes in water bodies; dyes which are discharged from textile industries pose a major threat to the environment due to its intense color and its carcinogenicity. A huge percentage of dye is lost from industries during the dyeing and printing process which hugely perturbs and causes eutrophication in the environment. Among the various methods that can be used for treating dyes, the most popular and economic is the photocatalytic degradation which ensures that the pollutant is not just transferred from one phase to another

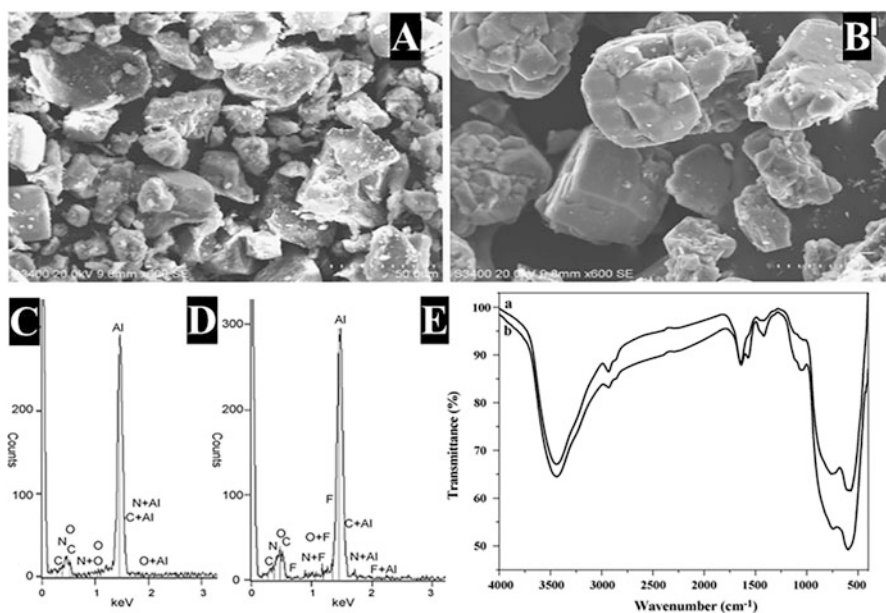


Fig. 2 SEM images of (A) Alumina/Chitosan composite, (B) Fluoride-adsorbed Alumina/Chitosan composite. EDAX spectrum of the (C) Alumina/Chitosan composite and (D) Fluoride-adsorbed Alumina/Chitosan composite. (E) Comparison of the IR spectrum of bare composite and fluoride-adsorbed composite. Reprinted with permission from (Viswanathan and Meenakshi 2010). Copyright Elsevier 2010. Reprinted with permission from (Elomaa et al. 2011). Copyright Elsevier 2011

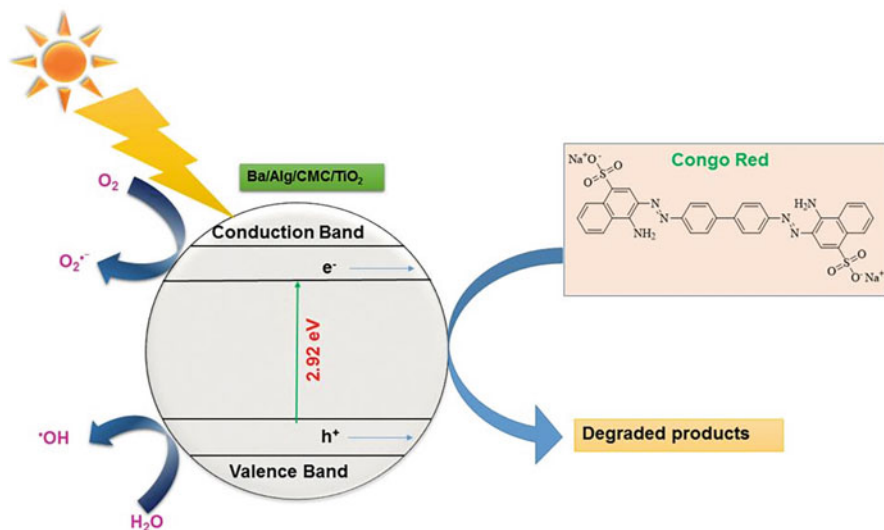


Fig. 3 Illustration of the mechanism of photocatalytic degradation of congo red. Reprinted with permission from (Thomas et al. 2016). Copyright Elsevier 2016

but degrades the dye molecule. The photocatalytic degradation implores on using highly effective and benign oxidation process for degradation using semiconductor materials. Among the semiconductor materials used, TiO_2 is the most commonly and effective semiconductor owing to its low cost, benign nature, high activity, and its chemical stability during irradiation. However, TiO_2 as a standalone material is difficult to recover thereby causing secondary pollution. This issue can be prevented by immobilizing it on biopolymers which are also environmentally benign among its other properties like its high surface area. A compatible pairing is the encapsulation of TiO_2 NPs in alginate-carboxymethyl cellulose composite gels and crosslinking with barium ion. This biopolymer composite was used for the degradation of congo red into various by products. The degradation ensued by the benzene ring breaking, -C-S- bond present the aromatic ring and sulfonate group breaking, the various -C-N and -C-C- bonds present in the chromophore groups breaking and the cleavage of the azo bond. The mechanism of photocatalytic degradation of Congo red is shown in Fig. 3 (Thomas et al. 2016).

Food Packaging

Food safety and packaging is a major concern that is being faced by the food industry currently. The short shelf life of food is a challenging issue and there have been many efforts to tackle this limitation. Food needs to be protected from any biological, chemical, and physical contaminations and kept fresh; however, there are major challenges as to which kind of packaging will be the best. Presently majority of the

food packing is based on synthetic polymers which are non-biodegradable and this causes major environmental concern. The cost of the packaging material also affects the total cost and the consideration of cheap and widely available eco-friendly materials is of huge interest. Biopolymers like cellulose and its derivatives, chitosan, alginates, etc. are some of the most abundant economical biopolymers available and their combination with other fillers have found huge success in food packaging and preservation industry. Chitosan has been used as a biomolecule-based films for packaging and has shown great promise because of its chelating ability and its antibacterial properties. The combination of chitosan with other fillers has also found be very advantageous in food packaging. An instance is the usage of PVA and chitosan that have been cross-linked by glutaraldehyde, the composite was used as both film and solution for food packaging. The composite solution was tested for preserving tomato as shown in Fig. 4, the composite film and solution was also tested for antimicrobial activity against *E. coli*, *S. aureus*, and *B. subtilis*, the chitosan-PVA film exhibited inhibition of 1.5, 1.2, and 1.4 cm against *E. coli*, *S. aureus*, and *B. subtilis*, respectively (Tripathi et al. 2009).

Another biopolymer that has been extensively used in food packaging industry is cellulose and its derivatives. Cellulose is one of the most abundant, sustainable, and eco-friendly biopolymers that is available. Cellulose can be derived from wood, plants, and it can also be extracted from organic waste of vegetables, stalks, fruits, stems, etc. Their usage in food packaging application also helps perturbs pollution and encourages recycling. Its mechanical properties also attract attention from textile industries, its thermal resistance enables it to shield food from UV rays. Furthermore, it has the ability to act as substrate for carrying antibacterial and antioxidant agents. These advantages have enabled cellulose and its derivatives to be crucial game changers in the food packaging industry (Liu et al. 2021). Despite these advantages, cellulose cannot function alone and it must be functionalized and these compatibility and combination has proved successful. A study that was carried out fabricated a composite from chitosan, carboxymethyl cellulose and ZnO NPs which provides antibacterial properties to the composite. The composite employed as a packaging film was able to store white cheese for 30 days at 7 °C, other properties like color, moisture, pH,

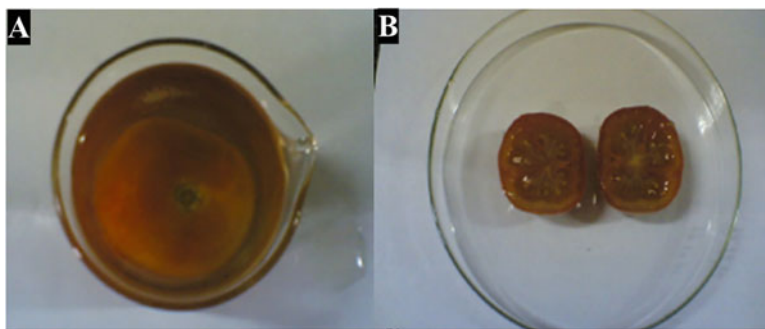


Fig. 4 Preservation of tomato in Chitosan–PVA solution: (a) as a whole and (b) into 2 pieces. Reprinted with permission from (Tripathi et al. 2009). Copyright Elsevier 2009

rheological properties were also tested. The composite film against pathogenic strain showed an inhibition of 5 to 15 mm and showed good influence on the total bacterial counts, yeast, mold and coliform. The prepared film was also compared against polystyrene packaging and showed good results (Youssef et al. 2016). Biopolymer-based composites have also been employed as edible films which can be consumed along with food. Biopolymers by virtue of its origin are mostly polysaccharides and proteins which are environmentally benign and are non-toxic to human beings. The purpose of adding edible biopolymer-based composite films in food is to increase the shelf life and food quality. The properties of composites that include antimicrobial, antibacterial, and antifungal are also added advantages and prevent the food from getting contaminated. The addition of edible biofilms also protect the food from UV radiation, moisture adsorption, and also increase the mechanical property of the processed food (Liu et al. 2021; Udayakumar et al. 2021).

Pharmaceutical Industry

Biopolymer composites are non-toxic, biocompatible, and influences cellular interactions for tissue development. Biopolymer composites by nature are similar to biological macromolecules that are present and utilized biologically. These qualities render biopolymer-based composites compatible to be used for biomedical applications. The most commonly used biopolymers range from polysaccharides like starch, alginate, chitosan, etc. to proteins like collagen, gelatin, and silk and microbial polymers like PLA, bacterial cellulose (BC), dextran, etc. Biopolymer composites can be constructed with two or more materials depending on the demands and the application to be used for. They can also be modelled to provide a suitable environment for growing and differentiation of cells. They also hold great advantage because of their high abundance and immunogenic potential. Biopolymer composites in this regard have been employed in a wide array of biomedical application, few examples that highlights the scope of biopolymer composites have been mentioned below.

Biopolymer composites have been used for wound dressing and there are also several commercial dressings like Xcell[®], Bioprocess[®], and Biofill[®]. Reports have shown that Bacterial-based coverings for wound treatment can reduce the pain, decrease infection and scarring and accelerate re-epithelization. The inclusion of metal NPs have also been found to assist in the regeneration, repairing of wounds, acts as oxidant, decreases inflammation, etc. These properties of NPs in composites resulted in a study where a Chitin/AgNPs composite hydrogel shown in Fig. 5 was employed for blood-clotting, and it was also studied for its antimicrobial activity (Kumar et al. 2010).

Biopolymer composites exhibit high bioactivity, flexibility and they can be molded to fit bone defects. These reasons including mechanical properties and osteoconductivity have influenced in the application of bone tissue engineering. Bone is made up of an organic phase and inorganic phase, similar to this biopolymer composites can be engineered where the inorganic phase can be incorporated to various polymer matrices particles and fibers. A study used calcium chloride and sodium

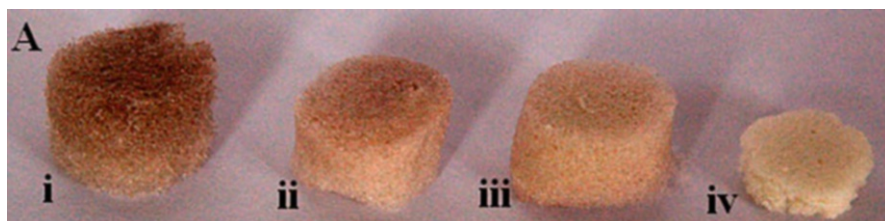


Fig. 5 Representative pictures of biopolymer composite with varying concentration of nanosilver; (i) β -Chitin +0.006% nanosilver; (ii) β -chitin +0.003% nanosilver; (iii) β -chitin +0.001% nanosilver; (iv) β -chitin control. Reprinted with permission from (Kumar et al. 2010). Copyright Elsevier 2010

carbonate to induce deposition of calcium carbonate on BC membranes which could mimic collagen nanofibers and promote biomineralization (Stoica-Guzun et al. 2012). There are other studies that have employed BC composites for bone tissue repair as well as cartilage replacement. A study performed evidenced that mechanical properties of BC/poly(dimethylacrylamide) double network gel resembled the cartilage that are present in the body and could be used to possibly replace artificial cartilage (Azuma et al. 2007). There has been major advances with BC-based materials in other medical fields as well with BC tubes used for replacing carotid artery in rats, the BC/carotid artery was found covered connective tissue indicating the success of BC as future replacement of blood vessels (Park et al. 2017).

Biopolymer composites have also been used as stent, an implanted device that is used for restoring normal blood flow. Biopolymer-based composites are safer compared to metal one since they are less obtrusive, biocompatible and biodegradable. The biopolymer composite stent consists of anti-restenosis which prevents toxicity and heals the blood vessels. Biopolymer composite has also been used as drug delivery systems; the functional groups present on the biopolymers enables in designing specific carriers that are either negatively or positively charged and interact biologically with the target sites. Chitosan has been found to have high affinity to cell membranes and due to its positive charge, it can be used for delivering a wide array of medicines via nasal, intravenous, oral, and ocular routes. Alginate-chitosan microsphere could also be prepared for delivering insulin, confocal images and schematic shown in Fig. 6a, b. The insulin showed higher loading and activity maintenance when it is loaded during the chitosan solidification. The *in vitro* release of insulin was investigated and found that the pH controlled how the insulin was released. The optimal release was in pH of blood environment, and it was found to be stable and sustained over 14 days shown in Fig. 6c with decreasing intensity of insulin with time (Zhang et al. 2011).

Automobiles Industries

The usage of plastics in automobile industries has become limited due to eventual exhaustion of cruel reserves worldwide and the environmental issues that are evident

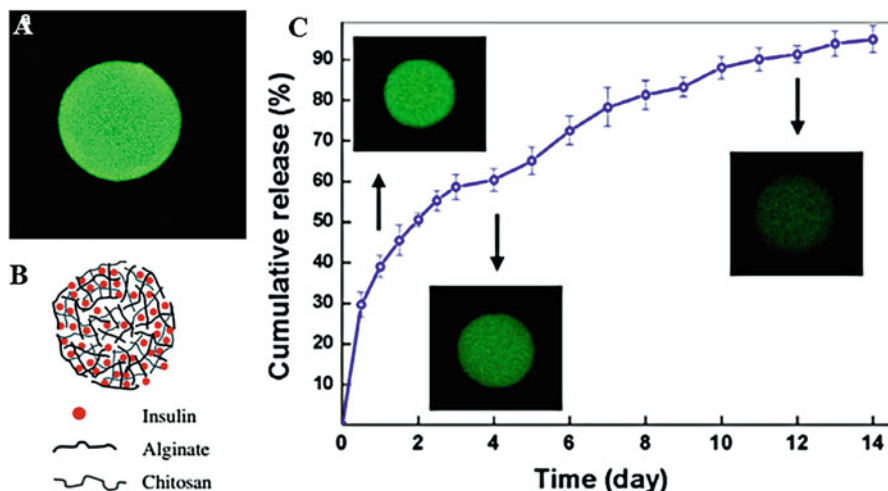


Fig. 6 (A) Confocal image of alginate-chitosan microsphere loaded with Alex Fluoresce-488-insulin. (B) Schematic image of the alginate chitosan microsphere loaded with insulin. (C) Confocal image of the microsphere with cumulative release of insulin over a span of 14 days with decreasing intensity of insulin in the microsphere. Reprinted with permission from (Zhang et al. 2011). Copyright Elsevier 2011

due to their usage. This concern has struck at the core of the automobile industries that are hugely dependent on plastics and synthetic fibers for their interior and exterior parts. Biopolymers and their composites have received renewed interest as substitutes for designing and fabrication of car parts based on plastics and glass fibers. Despite the long history and attempt of employing bio-based materials for commercial applications in the past by visionaries like Henry Ford (Fig. 7a shows henry ford testing a vehicle based on soyabeans composites) who started developing and employing composites derived from soyabeans for plastic-based car parts like a car foam shown in Fig. 7b. However, at that time the cost and concern of petroleum-based products outweighed the benefits that came with using natural fiber-based composites. But the eventual and increasing concern with depleting petroleum reserves and the pollution crisis, there has been revived interest in developing and commercializing composites based on natural fibers.

Over the years there has been huge leaps in the development of natural fiber-based products like kenaf and hemp, these materials are excellent candidates to replace glass fibers due to its low cost, low density, energy recovery, biodegradability, and CO₂ sequestration. These qualities they possess identify with the requirements the modern automobile industries are focused on. Automobile companies are hugely investing of materials with sound abatement properties along with reducing weight of vehicles for better fuel efficiency which can be increased to 75%. Natural fibers-based composites show excellent sound adsorbing properties, they are also lightweight, shatter resistant and enable the vehicle to save more energy. The mass of fiber-based composites car parts is not only lighter but their production also requires

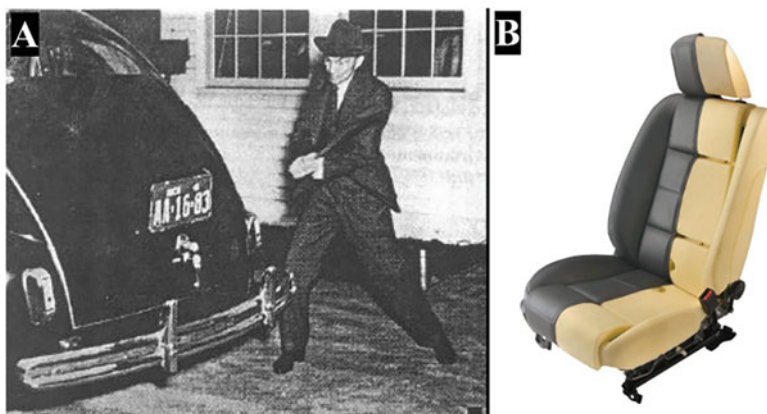


Fig. 7 (A) Henry Ford testing a car developed from soybeans composites. (B) A soy-based polyurethane foam introduced by Ford in 2008 for the Mustang

80% lesser energy. It takes around 23,500 British thermal units to produce a pound of glass fiber while it takes only 6500 British thermal units to produce a pound of kenaf fiber. There are also other added advantages of using natural fiber-based composites like its lower specific weight, easier processing and does not wear out tooling. There are several bast fibers that are at the forefront of replacing traditional fibers like hemp, flax, jute, and kenaf; other fibers like sisal, abaca, and banana leaves are also being considered. Natural fibers which were traditionally used only in making baskets, ropes, rugs, clothing, etc. and only employed as thermosets are now being intensively explored as thermoplastics. Their utility has been seen in various products ranging from household construction items like fencing, door profiles, siding, railings, furniture, etc. to automotive parts and even in marine components. In Germany, home to one of the most respected, innovative and competitive automobile industry in the world is leading in the usage of natural fiber composites. Its prestigious auto-manufacturers like Audi, BMW, Mercedes, and Volkswagen have started using natural fiber composites in both the interior and exterior parts of the automobile. One of the first example of commercialization of natural fiber composites was the inner door panel of 1999 S-class Mercedes-Benz made of 35% Baypreg F semi-rigid elastomer and 65% blend of hemp, flax, and sisal. There have been huge strides in the usage of natural fibers from other companies like Cambridge Industries that used flax/polypropylene composites in heavy trucks and rear-shelf trim panels. Dexter Corps makes sisal-based nonwoven mats for thermoforming. Georgia Composites' developed PP/sisal composites for boosting the performance of "Woodstock" -type materials. Mats and blends from hemp, flax, jute, and recycled polyethylene has also been produced by Kenex Hemp Ltd. There has also been tremendous contribution and collaboration between government agencies and industries to promote more sustainable materials like the collaboration between Pinnacle and U.S. Dept. of Agriculture in promoting agro-plastic. Several research institutions and industries across Europe are collaborating in the ECOFINA European project

aimed at substituting fibers and mineral fillers used in automotive industries with more sustainable materials and polymer matrix composites. These collaboration aims at producing auto components that are recyclable and lowering the weight of automobiles by using natural fiber-based composites which have lesser density to reduce the gas emissions from automobiles (Biagiotti et al. 2004; Mohanty et al. 2001).

Biosensing and Sensing

Biopolymers are three dimensional materials that are crafted by nature with origins from either plant or animal sources. The three-dimensional structure on biopolymers plays an integral role in their bioresponsive nature where they exhibit change in their properties and respond when they interact with other biological molecules like ATP, enzymes, glucose, antibodies, etc. The recognition of these biomolecules by biopolymers trigger events in the interaction and behavior of the biopolymers which are translated into responses that can be quantified and analyzed. The efficient analysis of interaction between the biopolymer and the analyte biomolecules is the heart in the development of biosensors. This can be achieved by effective attachment and immobilization of the biopolymer to the sensor interface. There are other attributes like the sensitivity toward pH and temperature that should be possessed by the biosensor. Although biopolymers possess many of the pre-requisites of biosensors, it suffers from limitations like low mechanical and chemical resistance. Biopolymers affinity toward water and its water absorbing characteristics also hinders their development as efficient biosensors. Biopolymers by virtue of their structures are poor electrical conductors, hence this also renders its viability as biosensors. In regards to all these limitations and to overcome them biopolymers are used for preparing composites with functional materials like carbon materials, metal oxides, enzymes, conductive polymers, etc. Biopolymer can also be used for designing molecularly imprinted polymer materials that is fabricated by polymerizing a functional monomer with a template or target molecule. The process termed as molecular imprinting in polymer matrices enables the formation of specific recognition sites. This property allows the polymer to highly specificity for the target molecule, where they can rebind to target molecules with same mechanism present between antibody and antigen.

Among the biopolymers, chitosan is the most widely used biopolymer for sensing and biosensing application due to its non-toxic, biocompatibility, bioresponsive and its fil-forming ability. The macromolecular chain of chitosan can also be modified with ease by conductive polymers and other functional materials for preparing composites with high specific surface area and electron transfer rate. The presence of amine and alcohol groups enables for easy immobilization of biomolecules like DNA, enzymes, and antibodies/antigens.

A study reports the fabrication of a DNA biosensor from $\alpha\text{-Fe}_2\text{O}_3$ microparticles and chitosan with terephthalaldehyde (TPA) as a bifunctional linker. The Fe_2O_3 microparticles impart high specific surface area and high electrical conductivity

while chitosan imparts the film stability. The amino groups of chitosan are covalently coupled with aldehyde groups of TPA on one end and on the other end of TPA the aldehydes react with modified amino of the DNA. $[\text{Fe}(\text{CN})_6]^{-3/-4}$ is used as the indicating probe with electrochemical impedance spectroscopy (EIS), and the biosensor was employed for measuring the hybridization capacity with good reproducibility and selectivity (Xu et al. 2015a).

Enzymes have also been incorporated with biopolymer composites, one such study is the inclusion of tyrosinase, a plant extract that was entrapped by polysaccharide-polysaccharide binding. The biopolymer composite was fabricated from biopolymers guar gum and agarose. The composite overcame the limitation of individual biopolymer like the brittleness of pure guar gum matrix. The two biopolymers are biocompatible and showed long-term stability of the developed sensor. The biosensor containing the enzyme forms a self-adhered layer on the working electrode and used for selectively and sensitively sensing phenols. Dopa and dopamine are detected by the reduction of the quinone species that are liberated biocatalytically. Despite the presence of enzyme which suffer from stability issues, the composite biosensor showed a low detection limit, reusability, pH, and storage stability (Tembe et al. 2006).

Aptasensors can also be developed from biopolymer composites. Evidence of this is the development of an electrochemical label-free sensor from a robust composite of MWCNT, ionic liquid and chitosan with a half-duplex hybridized capture-aptamer probe immobilized to the composite using glutaraldehyde (the schematic of the fabrication of the sensor is shown in the Fig. 8). Methylene blue was used as

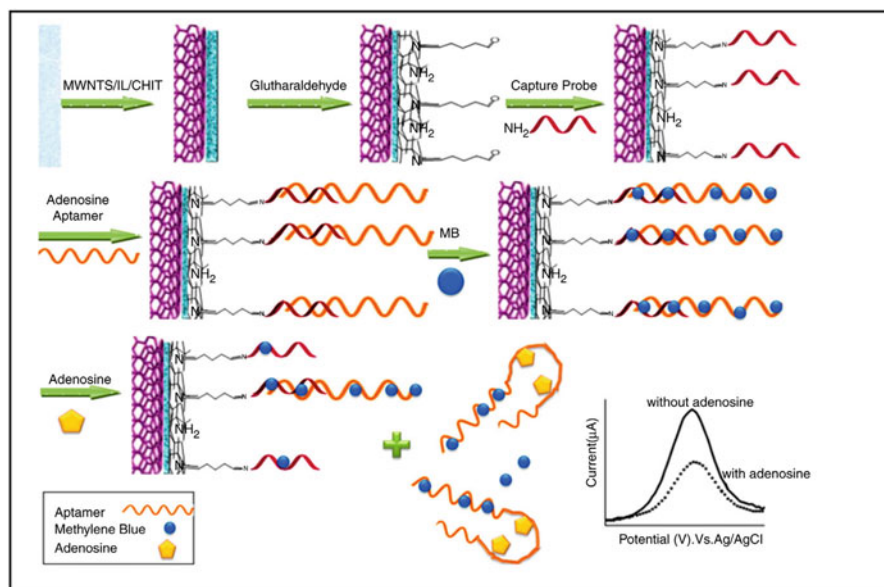


Fig. 8 Illustration of the fabrication of aptasensor from chitosan, MWCNT and ionic liquid. Reprinted with permission from (Shahdost-fard et al. 2013). Copyright Elsevier 2013

redox probe and the peak current intensity of the redox probe decreased with increasing adenosine concentration, these results due to the formation of aptamer-adenosine complex and displacement of aptamer from the electrode. The sensor had a low limit of detection and was successfully tested in blood serum and drug formation samples and showed excellent sensitivity toward adenosine in the presence of other interfering species such as cytidine, guanosine, and uridine (Shahdostfard et al. 2013).

Biopolymer-based composites have also been employed for development of bi-enzyme amperometric sensor for detecting organophosphorus pesticides. The amperometric sensor is fabricated from the electrodeposition of chitosan with carboxylated MWCNT entrapped, organophosphorus hydrolase and horseradish peroxidase. The sensor was employed for sensing prothiofos in the presence of H_2O_2 , the sensor could selectively detect prothiofos in the presence of interference nitrophenyl-substituted organophosphates because of pH and concentration of H_2O_2 (Stoytcheva et al. 2017).

Other than the studies mentioned here, there has been a wide range of sensors and biosensors that have been developed from biopolymer and its composites. They have been used for fabricating whole cell-based sensors, antibody-based sensors, aptamer-based sensors, nuclei acid-based sensors, enzymatic sensors, etc. The fast-paced advancement of biopolymer-based composites as sensors is the simultaneous advancement in the synthesis and tuning of biopolymer-based composites. The ultimate goal is the development of biopolymer composites as low cost, portable, fast data processing and reliable point-of-care-devices for detection of various organic and inorganic analytes (Karrat et al. 2020; Sawant 2017).

Energy Storage Materials

The energy crisis due to the depleting oil reserves is becoming more prominent and solutions are required. One of the possible pathways in easing this crisis is the development of more reliable and durable energy storage systems. Presently, majority of the commercial energy storage materials are based on heavy metals and non-renewable materials. There is a need for fabrication of more sustainable energy storage systems that are constructed from sustainable and renewable sources that can be disposed without causing any environmental damage. This requirement for sustainable energy systems has seen a surge in development of bio-based energy storage devices that are environmentally friendly from its source to its eventual disposing. Biopolymers as source for fabrication for sustainable energy storage devices is highly attractive due to its low cost, easy processing, renewability, environmental benignity and abundance. Although the advantages of employing biopolymers for energy storage devices are numerous, there are still issues due to its low electrical conductivity and their insulator properties. Among the biopolymers present majority of them are insulators and this hinders their viability as energy storage devices. There are several approaches when it comes to overcoming this advantage, the first is the conversion of the biopolymers into carbonized biomass to

increase its conductivity another approach is the inclusion of conductive materials and dopant. Biopolymers that contain redox active groups like quinones, flavins, and carboxylates show charge storage properties and with very little modification they can be used for the fabrication of energy storage devices as either supercapacitor or batteries. The attempts for developing sustainable batteries and supercapacitors began in the 2000s and there have been huge advancements since then. One of the breakthroughs in using biopolymer-based composites in energy storage devices is the development of polymer electrode from pyrrole and lignin. The presence of quinone in lignin enables it as proton and electron exchange and storage by redox cycling, this process when combined with the charge storage and conductivity of polypyrrole showed excellent charge properties. The two monomers are polymerized electrochemically to form interpenetrating networks and showed excellent scaling potential. The interpenetrating networks of the two polymers can also allow for the accommodation of smaller anions which can be pursued to increase its charge storage. It was found the addition of quinone groups to the interpenetrating network could enhance the charge storage properties, as such anthraquinone sulfonate was added to the polymer network and displayed an increased discharge capacitance of 147 mAh g^{-1} . This particular process opens up lot of avenues for enhancing the charge storage of the biopolymer composite by the addition of conductive materials like CNT, reduced graphene oxies, graphenes, metal oxides, small redox molecules, etc. (Nagaraju et al. 2014).

Other biopolymers composites have also been employed for the construction of energy storage devices. A common biopolymer that has been used for the fabrication of composites is cellulose. Cellulose is the most abundant biopolymer that has been used for other applications, however it does not possess redox active molecule. This makes cellulose an electrical insulator but its abundance in nature, cost efficiency, flexibility, light weight, wettability, and renewability has made them attractive biopolymers counterparts for fabricating energy storage devices. Cellulose, due to its insulating nature, has been paired with an array of materials that can impart electrical conductivity and enhance the charge storage property of the biopolymer composite. One of the most efficient methods of preparing a biopolymer composite with cellulose is the inclusion of conducting polymers in the composite matrix. An example of this success is the construction of a polymer paper battery from cellulose and polypyrrole (PPy), polypyrrole is coated thinly on the cellulose substrate. The material displayed a specific surface area of $80 \text{ m}^2 \text{ g}^{-1}$ and was employed for the construction of an aqueous battery (shown in Fig. 9) exhibiting a charge capacities between 38 and 50 mAh g^{-1} (Nyström et al. 2009).

Biopolymers are chemically composed of mostly hydrogen, carbon and oxygen, with carbon having much higher percentage composition than the other two. Due to this, biopolymers are an excellent source for the fabrication of carbon materials and raw materials for carbonization. Carbon materials prepared can exhibit various morphologies and properties depending on the raw material and the fabrication method employed. One of the most attractive carbon materials is porous carbon which have attracted significant attention due to its high electrical conductivity, electrochemical stability and specific surface area. High specific surface is highly

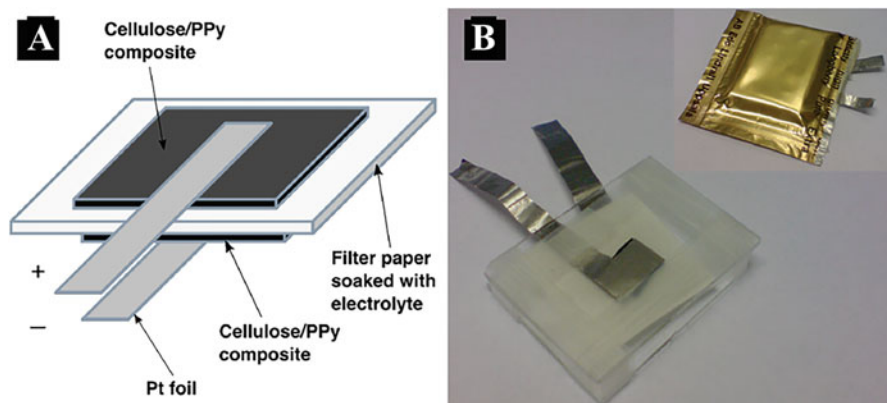


Fig. 9 (A) Schematic of polymer battery fabricated from cellulose and PPy composite. (B) Image of the fabricated cell with and without polymer aluminum pouch. Reprinted with permission from (Nyström et al. 2009). Copyright American Chemical Society 2009

sought after in carbon materials because of more active sites for storing charge. Hierarchical porous carbon is another type of carbon material with interconnected macro-meso-micro pores which enhances the ion adsorption and ion transport leading to increase rate capability and capacitance. Over the years, carbon-based materials have received a lot of attention and have been employed in various applications. Biopolymers like chitin, chitosan, cellulose, wood, lignin, etc. have been some of the most commonly used biopolymers for deriving carbon. Chitin, generally derived from seafood wastes can be sustainably used and one such study has employed for the fabrication of hierarchical porous carbon microspheres (shown in figure). The hierarchical porous carbon microspheres displaying a specific surface area of $1450 \text{ m}^2 \text{ g}^{-1}$ are employed along with polyaniline (PANI) nanoclusters. The composite employed for supercapacitor applications displayed a capacitance of 88 F g^{-1} with excellent capacitance retention (Gao et al. 2018).

The possibility of combination of more than one biopolymer with other materials for the construction of energy storage devices was also found viable. This was exhibited in a study where Lignin-resorcinol-formaldehyde (LRF) aerogels was paired with carbon aerogel using BC. The BC-LRF aerogel displayed a high gravimetric capacitance of 124 F g^{-1} . The aerogels of LRF and carbon, which are brittle are made superior and tougher by the addition of BC nanofiber network. The composite material activity makes it potential candidate for construction of flexible energy storage devices (Xu et al. 2015b).

Other than the few instances of employing biopolymer composites for the development of electrodes for supercapacitors and batteries. Biopolymer composites have also been utilized in the fabrication of separators for batteries due to its excellent wettability, high porosity, mechanical properties, flexibility, and low processing cost. They have also been employed for the fabrication of gel polymer electrolytes which is an interface between liquid electrolyte and solid electrolyte.

The characteristics of both the electrolytes are present in gel polymer electrolytes and they can act as both ionic conductors as well as insulators of the electrode. Biopolymer-based composites have also been employed as binders. Biopolymer composites for energy storage applications are still being studied but the versatility and multifunctionality of the materials offers huge scope in the future (Jabbour et al. 2013).

Concerns in the Sustainable Application of Biopolymer Composites

There are several challenges that need to be addressed for sustainable scaling-up synthesis and commercialization of biopolymer composites. The pairing issue of biopolymers with additives, poor moisture resistance, the tendency of the natural fibers to clump together, and low thermal stability are some of the major concerns. The growth and production of biopolymers and natural fibers are relatively easy and cheap and mostly done so by weavers, workers, and farmers. Natural fibers are grown depending on demand and can be grown quickly. Composite manufacturing on the other hand is more tedious and requires more capital investment, energy consumption, and specialized instrument/tools. Each composite manufacturing requires a different approach and usually different sources. Physical characteristics and behavior of biopolymer composites like fatigue, creep behaviors, moisture adsorption, and lifetime of biopolymer composites may vary depending on the source material and the physical conditions of location of the utilization of the biopolymer composites. The commercial production of biopolymer composites is still emerging and there is major focus in enhancing the processing and production. It may be noted that biopolymers despite being renewable and easily available, their processing, separation, and purification is another major challenge. This makes the supply, cultivation, transport, and storage of biopolymers complex; thus, a standard protocol is paramount to effectively utilize biopolymers, natural fibers along with the responsible waste treatment management. There is major research that is being concentrated on scalability, reducing the production cost and improving the yield and purity. The gradual advancement of the biopolymer industries has already resulted in the decrease of biopolymer prices.

As replacements for commercial materials and composites that are not sustainable, the performance and durability of the biopolymer composites should equal or be better than the material/composite it is replacing.

The challenge of recycling is another point that has been resounded frequently and stressed on. The recyclability of biopolymer and biopolymer composites although crucial is difficult to achieve, since the inclusion of recycled biopolymer and biopolymer composites may affect the properties and effectiveness of the overall composite. The recycled biopolymer or composite should be reconfigurable and replenishable to its pristine condition by treatment methods that also follow the themes of sustainability. This should be the key when designing biopolymer composites for application that are short lived. Another challenge with recycling is

the location at which the recycling plant is located to avoid extra expenses and manpower. Biopolymer composites as the name suggests contains two or more materials which may or may not be naturally derived. The extent and effectiveness of segregation of the materials is another crucial parameter when considering and designing recycling routes.

The understanding of the decomposing characteristics of biopolymer composites is also insightful when designing composites for particular applications. The conditions and time frame under which a biopolymer or natural fiber decomposes should be studied and analyzed critically. Establishing the accurate degradation properties of biopolymer composites is desirable and ensures that the degraded fragments do not affect the various biological pathways operating simultaneously in nature (Bari et al. 2021; Christian 2020).

Performance and Optimization of Biopolymer Composites

The careful and calculated choosing of biopolymer-based composites for a particular application ensures that the biopolymer performs well and is durable. Biopolymer-based composites are materials that react easily to environment, in many cases this is an added advantage but it can also be a hindrance in their performance in unfavorable environmental conditions. The location where the composite will be employed for different applications is an important parameter in the composite's performance. The decision of employing the composite where it will have maximum advantages is one of the most easy and apt methods in ensuring durability of the composite. The understanding of this dynamism between the composite and conditions under which it will perform is one of the most crucial steps in the success of implementing composites. The design of composites must be ensued according to performance criterions and standards for individual applications. The design of the composite should be accompanied with the aim of multifunctionality starting from its processing to its application. This multifunctional design of the composite would allow for fine tuning so as hasten its implementation. Systematic analysis of the variables that affect the composite and the influence it has in various applications, its performance and its durability for better decision-making should be focused in research.

Conclusion

This chapter gives a brief overview on biopolymer-based composites that is developed from a biopolymer reinforced with natural fibers, synthetic fibers, polymers, metal-based materials, other fillers, and dopants. Biopolymers-based composites by virtue of their raw materials and mode of preparation are designed with the intentions of renewability, sustainability, ease of manufacturing, biodegradability, lightweight, high specific area, durability, and easy tunability. The design of biopolymer-based composites is aimed at overcoming the limitations observed in individual materials and foster unique and useful functions not possessed by the either of the raw

materials. Most raw materials used in preparing composites can be obtained using simple methods, this promotes sericulture, farming, and waste management reducing pollution of all sorts and improves the overall quality of life. Biopolymer-based composites as evident from the studies are versatile materials and depending on the pairing of the materials and synthesis conditions, they can be employed in various industries like food packaging, automotive, medical, sensors, energy storage, and water treatment. They have huge potential and they are already being used in some already mentioned applications. The scaling and prospects of biopolymer-based composites are huge and can replace commercial materials that are petroleum-based or being deemed unsustainable. With the addressing of issues like durability in different environmental conditions, standard synthesis and assessment procedures and reducing the cost of production of biopolymers. Biopolymer-based composites have high chances in becoming more consistent materials and become an industry for replacing conventional materials that pose a threat to the well-being of the environment.

Cross-References

- [Biopolymer Films and Composite Coatings](#)
- [Biopolymers for Edible Films and Coatings in Food Applications](#)
- [Biopolymers from Renewable Sources](#)
- [Soy Protein Biopolymer](#)

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Abstract

Agricultural, industrial, and household waste is a huge threat to environmental sustainability in recent years. Plastics that are non-biodegradable are causing serious health issues also. Hence the development of biodegradable functional materials is a necessity in the current scenario. The quality of such materials is a major environmental concern, and the production of nanocomposites may decrease the impact of the problem. Biopolymer reinforced with nanofillers is a potential solution to the issue. Functional nanomaterials can be fabricated by the effective interaction between nanofillers and eco-friendly biopolymers. This interaction also enhances physicochemical features and biological properties. These biopolymer nanocomposites act as a substitute for conventional polymers due to their relatively better properties than pure biopolymers. This chapter

A. Paul (✉) · S. S. Sharma

Department of Chemistry, Sree Sankara Vidyapeetom College, Perumbavoor, India

compiles the details about biopolymers and their nanocomposites. The applications of biopolymer-based nanocomposites in various fields are also summarized.

Keywords

Biopolymer · Nanocomposite · Food packaging · Biomedical · Drug delivery · Tissue engineering · Sensing

Introduction

Nanoscience and nanotechnology have developed as a flourishing field in research in recent years expanding applications in agriculture, biomedical applications, energy production, cosmetics, pharmaceuticals, and diagnostics. Large surface area to volume ratio provided by nanosized materials enhances the properties. Not only pure nanoparticles but also nanocomposites have remarkable applications. Nanocomposites are hybrid materials obtained by combining two or more nanomaterials with different physical and chemical features. Their properties are much better than microcomposites.

Nanofillers can be dispersed in polymeric matrices in various quantities to obtain polymeric nanocomposites (Bustamante-Torres et al. 2021). Such dispersion will improve the mechanical and physical properties of the matrix. The features of the nanocomposite will be much better than the individual constituents in the matrix. Combining the features of polymer matrix and nanofillers results in a synergic effect, and high performance, unique nanocomposites can be produced. Polymeric nanocomposites possess excellent thermal, magnetic, electrical, optical, and mechanical properties which pave the way for a variety of applications (de Oliveira and Beatrice 2019). Polymer matrix and nanofillers are chosen according to the requirement in properties for various fields. Polymers may be categorized into biopolymers and synthetic polymers based on their origin. Biopolymers are obtained from animals, plants, and microorganisms. Since they are generated from renewable natural sources, they can replace synthetic polymers which have an origin from petroleum resources. They are exceptional biomaterials owing to their features such as biodegradability, lack of toxicity, cost-effective production, eco-friendliness, and biocompatibility. Also, they can be conveniently degraded by the action of microorganisms or inorganic compounds, which also reduce the possibility of environmental pollution caused by synthetic polymers (Folino et al. 2020). Thus, biopolymers such as polysaccharides, proteins, and lipids find applications in pharmaceutical, biomedical, and many other applications. Biopolymer nanocomposites are composed of two or more components, in which the bulk phase is a biopolymer, and the minor phase consists of nanofillers. This chapter discusses the most recent developments in research on biopolymeric nanocomposites for a variety of applications.

Another new generation material having wide applications is nanocomposite films. Such a film is produced by the combination of a biopolymer with a nanofiller and is processed to develop a film. In nanocomposite films, the major matrix is a

biopolymer and the dispersion of nanofillers in this matrix enhances the properties. Also, many limitations of biopolymers can be overcome by the addition of nanofillers. The addition of nanofillers can reinforce the matrix and create properties that are inherently absent in biopolymers. Some fillers are capable of improving the antibacterial and antimicrobial properties of the matrix which may assist in biomedical applications (Wrońska et al. 2021). It can improve the shelf life of food such as fruits, fish, vegetables, and so on in packaging applications also.

While considering food packaging applications, many synthetic polymers could satisfy all the requirements. Since these materials are non-biodegradable, they cause serious environmental pollution issues. A suitable methodology for the waste disposal of such synthetic polymers is not found till today. The increase in population always multiplies the usage of such synthetic polymers and their aftereffects happening worldwide over generations (Basavegowda and Baek 2021). Therefore, replacement of these polymers is a necessity, and current research is focusing on developing biopolymers that can reduce waste disposal. These biopolymers are degradable under biological decomposition without much affecting the sustainability of the environment. Their degradation produces harmless products which cause fewer disturbances to the earth's crust. Biopolymers are made of monomeric units combined by covalent bonds, and hence, these biopolymers may also be degraded by microorganisms via their enzymatic activities (Sorrentino et al. 2007).

Biopolymers can be derived from a variety of sources such as agricultural feedstock, industrial waste, animal and marine sources, polysaccharides, proteins, and so on. Biopolymers can be obtained from renewable and non-renewable natural sources. Natural biopolymers include polysaccharides, proteins, and lipids. Polysaccharides may be classified as wheat, pectin, chitosan, starch, and cellulose. Proteins and lipids include casein, collagen, soya, gelatin, and zein.

Even though, biopolymers act as potential candidates for food packaging, they are limited in use due to some disadvantages. They display poor mechanical and barrier properties which in turn affect their ability in processing. Industrial end-use applications are limited by the low heat distortion temperature, brittleness, and high gas permeability of biopolymers (Koh et al. 2008). These issues can be solved by the fabrication of biopolymer nanocomposites by adding nanofillers to biopolymer matrix. Mechanical, thermal, and physical properties can be enhanced by the incorporation of such nanofillers.

Earlier, major applications were completely occupied by the plastic industry. But, nowadays, bio-derived products are produced for such applications in an economical and sustainable way. Polypeptides and polysaccharides are utilized owing to their biocompatibility, biodegradability, sustainability, and minimum cytotoxicity (Wu et al. 2021). In the case of mechanical and thermal features, research is still going on to get the expected results. Industries always opt for petroleum-based polymers since they are cheap when compared to biopolymers. It exists as a threat to the sustainability of nature, and hence developing biopolymers in an economical strategy that is competitive with synthetic polymers is the future concern in this research area. Table 1 shows the source of biopolymers and their properties.

Table 1 Sources of biopolymers and their properties

No.	Source	Biopolymer	Properties	Reference
1	Crab	Chitosan	Good mechanical properties and antimicrobial properties less barrier properties	Sirisha Nallan Chakravartula et al. (2020)
2	Red algae	Agar	Good transparency and permeability, and high thermal stability	Mostafavi and Zaeim (2020)
3	Corn	Starch	Improved gas barrier features and good antimicrobial and antioxidant properties	Lu et al. (2009)
4	Fish skin	Collagen	Enhanced rheological features and high-moisture absorption	Lionetto and Corcione (2021)
5	Citrus peel	Pectin	Good chemical features, biodegradable, biocompatible, and edible	Espitia et al. (2014)
6	Milk	Casein	Nontoxic, biodegradable, and thermally stable	Chen et al. (2019a)
7	Cattle bone	Gelatin	Improved optical, mechanical, and barrier properties	Ramos et al. (2016)
8	Agricultural waste	Cellulose	Antimicrobial property, thermally and chemically stable, and crystalline	Wu et al. (2019)
9	Brown algae	Alginate	Aqueous solubility and flexibility	Katiyar and Tripathi (2019)
10	Soybean	Soy protein	Antimicrobial and gas barrier properties	Gautam et al. (2021)

Biopolymers

Biopolymers are considered as high-demand products since they can be easily degraded by microorganisms without adversely affecting the environment. Based on origin, biopolymers may be natural polymers or synthetic polymers (Muhammed Lamin Sanyang and Jawaid 2019). Natural polymers are obtained from natural renewable sources whereas synthetic polymers are obtained by polymerization or condensation reactions. Natural polymers include polysaccharides, proteins and lipids, and polymers such as polycaprolactone (PCL) and polylactic acid (PLA) are examples of synthetic biopolymers. Polymers such as polyhydroxybutyrate (PHB), polyhydroxyalkanoates (PHA), and polyhydroxybutyrate-valerate (PHBV) are produced by microorganisms like bacteria and are also considered as biopolymers (Mangaraj et al. 2019).

Among natural polymers, polysaccharides are produced from renewable sources, and they are made of monosaccharides linked by glycosidic bonds. Cellulose, starch, and chitosan are widely used natural polymers for different applications since they are cheap, non-toxic, and abundantly available (Sanyang et al. 2017). Among them,

starch consists of two varieties of D-glucopyranose including amylose and amylopectin (Mangaraj et al. 2019). It is generated from wheat, corn, and rice. Some disadvantages of starch being used as an efficient biopolymer are its high brittleness, high hydrophilicity, and difficulty in processing. Hence surface modification is a necessity to achieve the expected properties. It can be done by the addition of plasticizers or nanofillers. Such nanocomposites were found to have good tensile strength and mechanical properties. Another natural polymer widely used in various applications is cellulose. It is a biodegradable, hydrophilic polysaccharide comprising D-glucose repeating units and are linked by glycosidic bonds. It can be extracted from plants such as wood, cereal, and cotton. It is considered as a non-toxic, renewable, and easily available cheap biomaterial for packaging applications (Walzl et al. 2019). One of the drawbacks is its low water vapor barrier features. In order to improve mechanical properties, nanocomposites are formed by incorporating nanofillers such as metal and metal oxides such as copper oxide, zinc oxide, and so on (Anastasiou et al. 2014). The enhanced tensile strength can be obtained by the addition of silver nanoparticles along with better antibacterial properties. Shells of crabs, insects, and shrimps contain enormous amount of natural polymer, chitosan (Abdul Khalil et al. 2017). As in the case of starch and cellulose, chitosan is also biocompatible and biodegradable. It is the second most available polysaccharide and is made up of D-glucosamine and *N*-acetyl D-glucosamine. It is non-toxic and displays antimicrobial activity against yeast and bacteria. Even though, pure chitosan possesses good properties and enhancement in features can be obtained by adding plasticizers or nanofillers. Another animal-originated protein gelatin also finds immense applications in various fields. Denaturation of collagen results in gelatin and the features of gelatin are affected by the type of collagen (Roy and Rhim 2021). Copolymerization with other natural polymers such as chitosan and starch as well as incorporation of nanofillers can enhance various properties of gelatin.

Poly(lactic acid) is a synthetic aliphatic polyester obtained by the polymerization of lactic acid. It is a biodegradable polymer produced by fermentation of sources like rice, corn, and so on. It is an efficient biocompatible polymer having good mechanical strength and barrier properties. It is convenient to process to produce end use products. The brittleness of the polymer can be reduced by blending it with plasticizers or forming nanocomposites. Another commercially important synthetic biopolymer is polycaprolactone. It is a semicrystalline, linear, and hydrophobic polyester having good flexibility and elongation (Ortega-Toro et al. 2015). But poor mechanical and barrier properties limit its applications in packaging. In the case of polycaprolactone also, addition of nanofillers and formation of blends improves the properties.

Nanofillers

Novel trends and new perspectives that emerged in nanotechnology have created creative paths to facilitate the usage of nanofillers in various applications. Biopolymer nanocomposites cannot completely replace synthetic nanomaterials, but they can substitute plastic materials to a great extent (Saba et al. 2014). Nanofillers can

reinforce the biopolymer matrix thereby improving mechanical properties. Nanofillers have a size below 100 nm in any one of the three dimensions. They possess different shapes such as spherical, rod-shaped, needle-shaped, etc. The size and shape of the nanofiller greatly influence the features of the polymer matrix. Nano-sized materials will have a larger surface area which enhances the interaction of polymer matrix and filler. This betterment in interaction improves mechanical, physical, electrical, barrier, and thermal properties.

There are different types of nanofillers. They are organic, inorganic, clay, and carbon-based nanofillers. Organic fillers like cellulose, chitosan, and inorganic fillers like metals and metal oxides can act as efficient nanofillers. Fullerenes, carbon nanotubes, graphene, and graphene oxide are various carbon nanostructures that act as fillers (Youssef and El-Sayed 2018). Among the above nanofillers, clay is a cost-effective fine-grained material that can reinforce the polymer matrix. It can have a sheet or platelet-like structure. Different types of clay used as nanofillers are montmorillonite, halloysite, etc. Montmorillonite contains silica sheets attached to aluminum oxide sheets whereas halloysite is aluminosilicate with nanotubes having a cylindrical shapes. Halloysite possess good antimicrobial activity in addition to mechanical properties (Shankar et al. 2018). Among natural nanofillers, cellulose, chitin, and collagen are extensively used owing to the exceptional features such as easy availability, biodegradability, mechanical strength, and biocompatibility. Surface features can be improved by the reactive hydroxyl groups on the surface in the case of nanocellulose. Nanocellulose can be classified into three on the basis of crystallinity, size, and morphology. They are nanofibrillated cellulose, bacterial nanocellulose, and nanocrystalline nanocellulose. The difference in their structure, shape, and composition is due to variations in sources and synthetic methodologies. The functional groups present on the nanofiller also depend on the source. High surface area, non-toxicity, biodegradability, and biocompatibility of chitosan make it an excellent nanofiller in the production of various nanocomposites (Marín-Silva et al. 2019). But its use is limited due to poor solubility and stability in many applications.

Among inorganic nanofillers, metals and metal oxides are an important class that act as functional agents in reinforcement. They possess antibacterial and antimicrobial activity in addition to physiochemical properties. Copper nanoparticles are excellent candidates which exhibit antimicrobial properties. Similarly, silver nanoparticles display exceptional biological properties, and many research works have reported the applications in food packaging, drug delivery, wound healing, and biomedical applications. Besides metal nanoparticles, metal oxides like titanium oxide, zinc oxide, cerium oxide, aluminum oxide, copper oxide, iron oxide, etc., can also act as functional nanofillers. When exposed to ultraviolet radiations, reactive oxygen species in titanium dioxide assist in photocatalytic activity in many reactions (Juliet and Isaac 2015). Moisture penetration into the polymer matrix is prevented by the low hydrophilicity of titanium dioxide. Zinc oxide also shows antimicrobial activity which plays a good role in biomedical applications. Small particle size, high surface area, and UV- blocking effect of copper nanoparticles provide antioxidant and antibacterial properties for copper oxide nanoparticles.

Another class of nanofillers is carbon-based nanomaterials like fullerenes, carbon nanotubes, graphene, and graphene oxide. Significant electron mobility in carbon-

based nanomaterials creates excellent mechanical properties in the nanocomposite. Among these nanofillers, graphene has a high surface area as compared to others which enable good interaction with the matrix (Gan et al. 2017). But graphene has a tendency to agglomerate, and this limitation can be overcome by graphene oxide. Hence graphene oxide is the potential nanofiller among the carbon family. Graphene oxide has low solubility, and chemical modification and surface functionalization can help in improving solubility. Carbon dots are an emerging class in the field of biomedical applications as their biodegradability, photostability, and biocompatibility make them a promising material in different fields. They are quasispherical, amorphous nanomaterials having a size of less than 10 nm. Another advantage of carbon dots is that they are simple to synthesize and sources are easily available. They find many applications in cell imaging and sensing.

While optical properties are concerned, semiconductor quantum dots are gaining much attention in recent research works. Since they are very small in size, they have a great tendency to agglomerate and oxidize (Guo et al. 2016). Optoelectronic applications always choose graphene quantum dots-based nanocomposites due to their cost-effectiveness, availability, and biodegradability. Table 2 shows the properties of nanocomposites by the incorporation of nanofillers.

Table 2 Properties of biopolymer-based nanocomposites

No.	Biopolymer	Nanofiller	Properties	Reference
1	Gelatin	Clay	Improved mechanical properties than the pure polymer matrix	Baab and Zude (2008)
2	Chitosan	Silver/titanium dioxide	Excellent antibacterial properties enhanced shelf life in food storage	Lin et al. (2015)
3	Chitosan	Clay	Excellent mechanical properties, water vapor permeability is decreased	Lavorgna et al. (2010)
4	Poly(lactic acid)	Zinc oxide	Good antibacterial property	Materials (2019)
5	Poly(butylene adipate-coterephthalate)	Silicon dioxide	Excellent antimicrobial activity, Enhancement in mechanical and barrier properties	Venkatesan and Rajeswari (2019)
6	Starch	Clay	Increased stiffness and tensile strength	Sanyang et al. (2017)
7	Gelatin	Silver	Superior antibacterial and antimicrobial properties	Kanmani and Rhim (2014)
8	Starch	Silver/zinc oxide/copper oxide	Enhancement in mechanical properties	Peighambardoust et al. (2019)
9	Poly(lactic acid)	Zinc oxide	Better barrier and mechanical properties	Tang et al. (2020)
10	Chitosan	Curcumin	Water vapor permeability is reduced, and good antioxidant activity	Rachtanapun et al. (2021)

Synthetic Methodologies of Biopolymer Nanocomposites

Since the use of non-biodegradable materials is increasing day by day, environmental issues are also raising. For instance, the greenhouse effect is considered a huge environmental issue. So, it is high time to develop some functional compounds which act as alternatives for these synthetic materials. But the synthesis of eco-friendly products is very costly and it cannot be compared with synthetic materials in terms of cost. It is one of the limitations to use eco-friendly materials at a commercial level. While economic sustainability is concerned, the production of biopolymer nanocomposites at a cost comparable to synthetic polymers is a hot topic under discussion. In this matter, processing methodologies play a significant role. Both the cost and quality of the product are directly influenced by the synthetic strategy. Usually, biopolymer blends or nanocomposites are developed for improving the features. Methods such as electrospinning, solution casting, ultrasonication, etc., are used to fabricate nanocomposites. Very thin nanofibers can be developed by the process of electrospinning. The spinning technique can improve both the thermal and mechanical properties. Bio nanocomposites are developed by mixing the biopolymer and nanofiller and then heating and stirring till we get a homogenous suspension. It is dried out to get nanocomposites by means of solution casting. Another method of synthesis is ultrasonication which is a more sophisticated technique to disperse the nanofiller in the polymer matrix without agglomeration. The uniform distribution of the nanofiller in turn improves the properties of the material also (Abral et al. 2018).

Properties of Biopolymer Nanocomposites

Many polysaccharides act as a matrix to form biopolymer nanocomposites. Among them, chitosan, starch, and hyaluronic acid are widely used. Tissue engineering is one of the major applications in which biopolymers find their use. Biodegradability, oxygen permeability, water sorption, and blood coagulation of chitosan make it an efficient material in scaffolding. When chitosan is composited with nanoclay, it was found that there was a significant improvement in mechanical properties owing to the effective interaction and uniform dispersion of nanoclay in the chitosan polymer matrix (Cano et al. 2017). Such a biodegradable nanocomposite was used as a packaging material. Similarly, biofilms can be fabricated by mixing bacterial cellulose in hyaluronic acid for healing wounds. It was found that the incorporation of cellulose improved roughness and thermal stability. These properties assist the material in tissue engineering applications (de Oliveira et al. 2017). In another work, cellulose is dispersed in alginate by means of solution casting. There was a significant improvement in tensile strength in addition to the decrease in water vapor permeability and water solubility. In another work, cellulose dispersed in starch and as a result, thermal and mechanical properties are improved when compared to pure starch matrix. Uniform dispersion and excellent interaction of cellulose in starch are found to be the reason for the enhancement in the properties (Ghanbari et al. 2018).

Collagen and gelatin are also considered as a potential biopolymers which can act as an excellent matrix for the formation of nanocomposites. Polylactic acid is another renewable biopolymer which finds applications in various fields.

Applications of Biopolymers

While biopolymer acts as the raw material to produce different end use products, the features enhanced by the incorporation of nanofillers decides the design of each application (Vlăsceanu et al. 2019). For example, mechanical properties are necessary for developing biofilm for packaging applications, at the same time antimicrobial activity is essential for biological applications like drug delivery and tissue engineering. Hence, enhancements in specific properties needed to be targeted to reach the final aimed product. In the case of 3D printing thermomechanical factors play a key role. In such cases, proteins are added as plasticizers to increase the thermal stability.

Among various applications, the most important one which needs biodegradable biopolymer as a raw material is packaging. The material for such a purpose should be cost-effective, easy to decompose and recycle, biodegradable, and easy to transport. Research is still continuing to develop a raw material that can satisfy all the above features. There are inventions at the laboratory level. But the material still needs optimization to take it to commercial level. Biopolymer nanocomposites are widely used in biomedical applications also. Tissue engineering, drug delivery, and wound healing are a few biological applications. Biopolymer nanocomposites are also utilized as supercapacitors since the potential of electrode material can be improved by using nanofillers. Specific capacitance can be enhanced by the usage of biopolymers like lignin. Similarly, carbon nanomaterials like graphene, carbon nanotube, and carbon fibers find applications in the field of supercapacitors (Okonkwo et al. 2017).

Packaging

Efficient packaging is a necessity in the food industry. Good packaging provides expansion of the shelf life of food materials against decay via chemical, microbial, or any other hazards. It also retains safety and maintains quality (Cakmak and Sogut 2020). These packaging materials should maintain food quality and are to be convenient for processing till it reaches end-use product. Usually, materials such as glass, metal, plastic, or paper are used as packaging materials. Among plastics, petroleum-based materials are mainly used owing to their lightweight, availability, flexibility, good mechanical properties, and low cost (Wang et al. 2019). But the disadvantage of these petroleum-based packing materials is their non-biodegradability. Some of them take more than 15 years to degrade which is a severe issue while environmental sustainability is concerned. These polymers are a severe threat to nature by causing soil and water pollution. Due to this drawback, there is a high demand for eco-friendly

biopolymers with good mechanical properties for the food industry. Excellent biocompatibility and biodegradability make them ideal candidates for this purpose. While using biopolymers as packaging materials, waste disposal and composting them are comparatively easy and safe since they can be degraded by microorganisms in the soil (Abdul Khalil et al. 2017). High price, low mechanical, barrier, and processing properties limit the use of biopolymers. But it is a challenge to develop biopolymers that can compete with the mechanical and barrier properties of petroleum-based polymers. Economical production cost is also a major criterion in packaging applications.

Structure modification of biopolymers via copolymerization and nanocomposite fabrication are the methodologies to attain these expected properties. In some cases, plasticizers are added to the biopolymer to enhance the properties. These plasticizers can exchange intermolecular bonds thereby strengthening the polymer chains (Mulla et al. 2021). They also provide flexibility by creating variations in conformation and hence brittleness of the polymer can be reduced. Glycerol, triacetin, sorbitol, and polyethylene glycol are used as plasticizers in the food industry. The quantity of plasticizers should be controlled since their over usage may cause health and environmental issues. Copolymerization is also a suitable method to attain properties. Compatibility, uniform dispersion, and miscibility have to be checked while copolymerization is done. Another method to improve the properties of biopolymers is nanocomposite formation by dispersing nanofiller in the biopolymer matrix (Zhong et al. 2020). It is a thrust area of research in recent years and studies prove that water and gas barrier properties can be improved along with mechanical properties by developing nanocomposites. This in turn increases the shelf life of packed foodstuff since many of the nanofillers possess antimicrobial and antibacterial properties.

For safe and secure food transport, efficient packaging of food is necessary. For this purpose, reducing microbial growth is essential. Antimicrobial packaging will inhibit the growth of microorganisms to a great extent so that shelf life can be improved (Kumar et al. 2018). Furthermore, the quality of the food can be maintained with a good appearance, and eventually it can reduce wastage. Organic materials like polymers, acids, and enzymes, and inorganic materials like metal oxides and metals can be used as antimicrobial agents. Other examples of antimicrobial agents are essential oils, peptides, and plant extracts. Metal nanoparticles like zinc, copper, and silver display antimicrobial activity. Zinc oxide, titanium oxide, and copper oxide are examples of metal oxides possessing the same property. There are research works reported for chitosan-based nanocomposites utilized in the packaging industry (Nonato et al. 2019). It is found that chitosan-based films could inhibit the multiplication of microorganisms. Like any other biopolymer, temperature, molecular weight, concentration, and type of microorganism affect the activities of microorganisms. The incorporation of metal oxides into chitosan-based films improves the antimicrobial property.

Reducing the growth of microorganisms and lipid oxidation are the ways to improve the shelf-life of food materials. By delaying the process of oxidation, better maintenance of food quality may be obtained. Prevention of food oxidation by the

use of antioxidants is one of the methods to improve the same. Both synthetic antioxidants and natural antioxidants can be used to preserve food. Examples of natural antioxidants are curcumin, catechin, quercetin, mint, oregano, cinnamon, and so on (Vilela et al. 2018). In many biopolymers, these antioxidants are incorporated to get the expected results. Based on the action of antioxidants, they can again be classified as primary and secondary antioxidants. Secondary antioxidants are able to absorb ultraviolet radiation thereby preventing oxidative effects on the food products. Benzophenones, zinc oxide, titanium oxide, etc., are examples of such secondary antioxidants (Goudarzi et al. 2017).

Biopolymer-Based Nanocomposites for Drug Delivery and Wound Healing Applications

Biopolymer-based nanocomposites have been widely employed in drug delivery systems due to their biocompatible and biodegradable nature (Azmana et al. 2021; Kurakula et al. 2020). They have been used in different routes of drug delivery and have revolutionized the field of drug delivery by improvising the routes as well as vehicles for drug delivery.

Cellulose and Cellulose Derivatives

Bacterial cellulose derived from bamboo and sodium alginate was employed in developing a hydrogel nanocomposite system for controlled protein delivery by Li Ji et al. Bacterial cellulose was obtained by the inoculation of *Acetobacter xylinum* employing Moso Bamboo enzymatic hydrolysate as a carbon source and different weight % (0.25, 0.5, 0.75, and 1.0) of sodium alginate (SA) was added at the time of inoculation so as to obtain the nanocomposite hydrogels. The morphological analysis revealed the formation of interconnected porous structure and the pore size increased with increasing concentration of sodium alginate. The maximum pore size (50–70 nm) and uniformity in pore distribution was attained with 0.75 SA added hydrogel and at 1% SA concentration, the interconnected porous structure disappeared resulting in a structure with line-shaped nanofibrils. The drug release mechanism and pH responsiveness of two model drugs namely bovine serum albumin (BSA) and lysozyme (LYZ) entrapped in the nanocomposite hydrogel were evaluated. The results of the study indicated that LYZ has a better pH-dependent releasing ability as a result of electrostatic forces of adsorption between hydrogel and LYZ. However, due to hydrophobic adsorption between BSA and hydrogel, the drug release of BSA was poorer compared to LYZ. Overall, the study demonstrated that the hydrogel nanocomposites based on SA–BC are promising in delivering hydrophilic drugs such as proteins (Ji et al. 2021).

Nusheng Chen et al. synthesized nanocomposite hydrogels based on modified carboxy methyl cellulose (CMC) and diblock copolymer as hydrophobic micelle cores for controlled and localized release of hydrophobic drugs. The modification of CMC was done by hydrazide and aldehyde and the micelle core was based on a copolymer system which is pH-responsive, poly(ethylene oxide) (PEO) -block-poly

(2-(diisopropylamino) ethyl methacrylate (PDPA). The injectable nanocomposite hydrogel was obtained by dissolving the two modified CMC precursors in a micelle solution loaded with drug and then co-extruding with the help of a double barrel syringe. The cross-linking in hydrogel composites is facilitated through a Schiff base reaction. Nile red was chosen as a model hydrophobic dye to test the efficiency of the copolymer micelle's loading ability since it will exhibit fluorescence only in hydrophobic environments. The in vitro release of doxorubicin was also evaluated in simulated body fluids having physiological conditions. The hydrogel composites based on CMC and PEO-b-PDPA micelles exhibited negligible cytotoxicity and was successful in slow and controlled drug release which can be tuned by varying the pH as well as by varying the hydrogel precursor concentrations (Chen et al. 2019b).

In order to improve the mechanical properties of hydrogels of carboxy methyl cellulose (CMC), carboxy methyl β -cyclodextrin (cm- β CD) was cross-linked with CMC to form a hydrogel composite. The incorporation of cm- β CD has significantly improved the mechanical performance of the hydrogel as well as the storage modulus and swelling properties. The drug release study conducted using Tetracycline (TC) as a model drug revealed that the CMC- cm- β CD hydrogel was able to release TC in an effective manner both at high and low TC loading. The effective release of TC after crosslinking with cm- β CD is due to the complexation of the drug with cm- β CD. The antibacterial activity tested using *S. aureus* bacteria was also found to be better in the case of CMC- cm- β CD hydrogel loaded with 50 mg TC (Jeong et al. 2018).

J.T Orasugh et al. employed cellulose nanofibrils (CNF) isolated from jute fiber for the preparation of nanocomposite film with hydroxy propyl methyl cellulose (HPMF) and tested for transdermal drug delivery of an analgesic Ketorolac tromethamine (KT). The in vitro release studies of KT revealed that the drug was released from the composite film in a controlled manner for a prolonged time period of 8 h. The precise release of KT from the HPMF-CNF was ascribed to the three-dimensional structure and less swelling property of CNF (Orasugh et al. 2018).

Chitosan

Chitosan mainly seen in crustacean shells of marine organisms is another widely used biopolymer for developing drug delivery systems with improved biocompatibility and biodegradability. The free amino groups present in the chitosan backbone help in encapsulating hydrophobic drugs and also help in the incorporation of proteins and peptides which are negatively charged. The ability to get fabricated into various structures and shapes makes chitosan adaptable for performing different functions in drug delivery systems (Tao et al. 2021).

Deformable liposomes (DL) based on chitosan loaded with flurbiprofen was proposed by Chen et al. as an ocular delivery system for delivering flurbiprofen with a view to overcome conventional eyedrops. The coating of chitosan on DL improved the penetration as well as absorption leading to greater bioavailability of the drug in the ocular region. Moreover, the chitosan-coated DL showed reduced ocular irritation of flurbiprofen and also favored the biocompatibility of the drug in the ocular region (Chen et al. 2016).

An effective transdermal (TD) drug delivery option based on chitosan modified with glycidyl and butyl methacrylate and hyaluronic acid was developed by

Anirudhan et al. for lidocaine (LD) delivery directly into the systemic circulatory system. The TD device was tested for *in vitro* release and *in vivo* skin adhesion as well as skin irritation assay. The results indicated that the transdermal patch performed controlled release of lidocaine in a sustained manner with improved antimicrobial activity and with no skin irritation (Anirudhan et al. 2016).

In another study, the inclusion complex of amlodipine besylate (APB) and β -cyclodextrin was prepared by two different methods, namely kneading method and microwave method initially, and then mixed with a nanocomposite of chitosan-graphene oxide and sodium alginate by microwave-assisted synthesis. The nanocomposite containing inclusion complex was further analyzed for drug release kinetics by varying the pH using six different kinetic models. The best-fitting kinetic model was selected on the basis of the correlation coefficient (R^2). Accordingly, the influence of relaxation and diffusion on the drug release process was explained using the Peppas-Sehlin equation (Khushbu and Jindal 2021).

A nanocomposite hydrogel based on modified chitosan (modified with methacrylated glycol-MeGC) and montmorillonite (MMT) for bone tissue engineering application was proposed by Cui Z. K et al. The MMT was introduced into MeGC hydrogel systems and was photopolymerized using a photo initiator (riboflavin) to obtain an injectable bone regeneration biomaterial with improved osteoconductive nature. The incorporation of MMT in the hydrogel helped in the formation of a microporous structure having interconnectivity and better mechanical stability. The *in vitro* studies showed better proliferation and differentiation of mesenchymal stem cells. The *in vivo* study conducted using a mouse calvarial defect model showed that the nanocomposite hydrogel can employ new cells and can also help in calvarial healing indicating its potential in tissue engineering and regenerative medicine field (Cui et al. 2019).

A piezopolymer nanocomposite system based on chitosan and hydroxylated barium titanate (BaTiO_3) was reported for tissue engineering by Prokhorov et al. The nanocomposite exhibited biocompatibility with fibroblast cells and also the presence of BaTiO_3 does not cause any cytotoxicity issues as revealed by the cell viability. Hence, the nanocomposite film can be used as a flexible platform for cell proliferation and also for skin regeneration (Prokhorov et al. 2020). A brief list of other bionanocomposites based on chitosan and the relevant application is listed in Table 3.

Silk Fibroin

Silk fibroin (SF) based nanocomposites are widely employed for tissue engineering mainly for repairing nerves, cartilage, and skin due to its superior mechanical properties, biocompatible nature, and its ability in producing lower immunogenic responses. Y. Wang et al. reported sodium alginate (SA) and silk fibroin (SF) composite hydrogel for the delivery of bovine serum albumin (BSA) and tetracycline hydrochloride. The hydrogel composites were made flexible by improving the crosslinking between SA and SF employing a carbodiimide. The composite hydrogel's swelling and mechanical properties as well as their gelation time, biodegradation, and biocompatibility were evaluated. The hydrogel composite exhibited a sustained release of micro and

Table 3 List of chitosan-based bionanocomposites and applications in the biomedical field

Composite system	Synthesis method	Application	Reference
Chitosan, cellulose, and hydroxyapatite	Solvent exchange method	Bone tissue engineering	Synthesis et al. (2021)
Chitosan and iron oxide	Green synthesis	Antibacterial and antioxidant activity	Bharathi et al. (2019)
Chitosan, reduced graphene oxide, and iron oxide	One pot synthesis-solvent casting	Hyperthermia treatment	Barra et al. (2020)
Chitosan and cellulose loaded with betamethasone/silver sulfadiazine	Solvent casting	Wound dressing	Riccio et al. (2021)
Chitosan and cellulose encapsulated with 5-Fluorouracil	Ionic gelation using a crosslinking agent (sodium tripolyphosphate)	Treatment of colorectal cancer	Yusefi et al. (2021)
Chitosan and silver nanoparticles	Green synthesis	Antibacterial agent and drug delivery	(Saruchi et al. 2022)
Chitosan and graphene oxide	Gelation method	Metronidazole oral drug delivery	Kumar et al. (2021)

macromolecular drugs with no cytotoxicity and also showed appreciable biodegradation within 14 days duration (Wang et al. 2021).

A ternary nanocomposite scaffold based on silk fibroin, carboxy methyl cellulose, and magnesium hydroxide nanoparticles was envisaged for wound dressing applications by R.E. Keihan et al. The hybrid composite showed to be promising for wound healing applications as revealed by the in vivo assay results for wound healing with 82.29% healing in 12 days. The composite also showed good biocompatibility and higher bactericidal activity recommending the possibility of the compound in diverse biomedical applications (Eivazzadeh-Keihan et al. 2021).

Another group reported chitosan nanoparticle-incorporated silk fibroin hydrogel composite for delivering dexamethasone sodium phosphate (DEX). For preparing the drug release device, the DEX was incorporated in chitosan nanoparticles by gelation (ionotropic) and then it was transferred to SF solution and sonicated to induce gelation. The in vitro drug release studies indicated that the chitosan nanoparticles helped in encapsulating the DEX for controlled release of the drug while SF acted as a matrix for prolonging the drug release (Akrami-Hasan-Kohal et al. 2021).

Biopolymer-Based Nanocomposites for Tissue Engineering Applications

Tissue engineering aims to develop functional constructs by making use of a scaffold, functional tissues, cells, and other biologically relevant molecules with a

view to restore or maintain damaged tissues or whole organs (Zhao et al. 2020). Biopolymers and their nanocomposites are frequently being used as scaffolds since they can meet the prerequisites of a scaffold material such as porosity (to endorse vascularization and integration of tissues), structural resemblance with bioactive molecules, biocompatible, and controlled biodegradation (Islam et al. 2020).

Bacterial cellulose (BC) is commonly employed for tissue engineering applications compared to plant cellulose on account of the ease of production, purity, and adaptable nature. The slowly degradable nature of BC can be altered by the addition of other components. The modification can be done either at the time of synthesis of BC or post-synthesis using other biomaterials such as collagen, hydroxy apatite, hyaluronic acid, pectin, etc., Z. Keskin et al. prepared BC composite modified with keratin for tissue engineering of the skin. The main reason for the addition of keratin was to increase the bonding of fibroblasts of skin on the surface of BC. Cytotoxicity of the composites was checked using mouse fibroblast cells (L929) and also by MTT assay. The BC-keratin composites exhibited appreciable viability and biocompatibility and also showed better adhesion of fibroblasts. The group has studied both in situ and post-modified keratin-BC composites, and both composites were found to be effective for use in dermal tissue engineering applications (Keskin et al. 2017).

Conductive scaffolds based on cellulose and polypyrrole (Ppy) for nerve regeneration have been reported by R. Elashnikov et al. The surface of cellulose acetate butyrate nanofibers obtained by electrospinning was modified using polypyrrole (Ppy) and was used for the preparation of scaffolds. The coating of Ppy on cellulose helped in improving the conductivity of the scaffold for use in tissue engineering of neural tissues. The cytotoxicity of the composite fibers by LDH assay also revealed the non-toxic nature of the scaffold even after Ppy coating. The adhering of Ppy particles on the cellulose surface enhanced the roughness and subsequently helped in promoting the adhesion of human neural cells (SH-SY5Y) and also increased the viability of SH-SY5Y cells up to a period of 15 days (Lyutakov 2019).

The approach of electrical stimulation for nerve regeneration was adopted by another group (Zha et al. 2020). Cellulose and two conducting polymers (poly (N-vinyl pyrrole (PNVPY) and poly (3-hexyl thiophene (P3HT) were made into a nanofibrous mat by polymerization (in situ). The thickness as well as the porosity of the composite mats was superior to electrospun cellulose mats which helped in improving the adhesion of PC12 cells. The application of electrical stimuli augmented the proliferation of PC12 cells and the fibrous mats also showed excellent biocompatibility.

Waghmare et al. suggested the chemical alteration of carboxy methyl cellulose by the introduction of sulfate groups for constructing injectable scaffold tissue engineering cartilage. The macroporous nature of the scaffold helped in the chondrogenesis of mesenchymal stem cells as a result of the binding of growth factors through ionic interactions. The highly porous nature with and strong pore wall, the injectable scaffolds showed good resilience and were able to retain their initial shape making them suitable for engineering the load-bearing tissues (Waghmare et al. 2018).

Table 4 Synthesis methods and tissue engineering applications of biopolymer nanocomposites

Components of composite	Synthesis method	Application	Reference
Carboxymethylcellulose, hydroxy apatite, and gelatin	Freeze drying followed by crosslinking	Bone tissue engineering	Karvandian et al. (2020)
Carboxymethylcellulose and polyethylene glycol	Solution casting	Skin tissue engineering	Zennifer et al. (2021)
Bacterial cellulose, β -glucan, graphene oxide (GO), and hydroxy apatite	Free radical polymerization & freeze drying	Bone tissue engineering	Umar Aslam Khan et al. (2021)
Collagen and tricalcium phosphate	3-D printing technique	Hard tissue regeneration	Kim et al. (2017)
Starch nanocrystals and gelatin	3-D printing technique	Cartilage tissue engineering	Piluso et al. (2019)

Poly(butylene succinate) (PBS) and cellulose nanocrystals (CNC) bionanocomposites as the scaffold was prepared by electrospinning for tissue engineering application by Huang et al. Thermal and mechanical properties as well as the porosity of PBS/CNC scaffold were found to be superior at 3 wt% composition of CNC. The scaffold exhibited appreciable biodegradation compared to neat PBS and the cell viability of fibroblast cells (3 T3 cells) remained 100% even after culturing for 7 days suggesting good biocompatibility. The incorporation of CNC helped in improving cell proliferation and biodegradation of the scaffold (Huang et al. 2018).

A nanocomposite of chitosan incorporated with gold, hydroxyapatite, and graphene was prepared by hydrothermal method and was cast into a film using poly vinyl alcohol (PVA) by gel casting technique. The polymeric film showed significant differentiation and viability of osteoblasts and was also effective against both strains (gram positive and gram negative) of bacteria indicating its potential in the field of bone tissue engineering applications (Prakash et al. 2020).

The various strategies and the composition of biopolymer composites employed in tissue engineering applications are given in Table 4.

Biopolymer-Based Nanocomposites for Biosensing Applications

Biopolymer nanocomposites have also been recognized in the field of sensing especially biomolecules such as enzymes, nucleic acids, antibodies, proteins, enzymes, etc., and also other chemical species. Due to the unique biocompatible nature of biopolymers, several sensing technologies with high selectivity and specificity have been devised based on the composites of biopolymers. Biosensors are used in the detection of biomolecules and the detection is made possible by visualizing the change in optical, chemical, mechanical, or thermal properties (Subhedar et al. 2021).

The detection of purine bases such as adenine and purine employing cellulose (NC) and CNT nanohorns (SWCNHs) was reported by Ortolani et al. A thin film

comprising of NC and SWCNHs with higher sensitivity and selectivity was fabricated and the detection was done using linear sweep voltammetry (LSV). The fabricated electrode demonstrated a lower detection limit (LOD) of $1.5 \times 10^{-7} \text{ mol L}^{-1}$ for guanine and $1.9 \times 10^{-7} \text{ mol L}^{-1}$ for adenine. The electrode was also successful in the real-time monitoring of purine bases in human serum and fish sperm (Ortolani et al. 2019).

An enzymatic biofuel cell was developed for the selective detection of glucose using bacterial cellulose (BC) modified with gold nanoparticles (AuNP) as the electrode material. The synergic combination of BC and AuNP in transferring the electrons from the enzyme (glucose/ O_2) to the surface of the electrode helped in achieving a lower LOD value ($2.874 \mu\text{M}$) and a broader detection limit ($0\text{--}50 \text{ mM}$). The biofuel also exhibited a higher value of power density ($345.14 \mu\text{W cm}^{-3}$) indicating its possibility to be used as a self-powered biosensor for selective detection of glucose (Lv et al. 2018).

Hemoglobin impregnated on GCE modified using a nanocomposite of cellulose microfiber and graphene (GR-CMF) was used for quantifying hydrogen peroxide (H_2O_2). The electrode showed good electrochemical behavior in the detection of H_2O_2 even in the presence of other interfering compounds with a selectivity of ($0.49 \mu\text{A} \mu\text{M}^{-1} \text{ cm}^{-2}$). The biosensor also exhibited appreciable stability under operating conditions, lower LOD value (10 nM) and can be used for the detection of H_2O_2 in biological as well as pharmaceutical models (Velusamy et al. 2017).

Detection of glucose by in vivo (oral) and dermal monitoring was reported by C. T Tracey et al. using hybrid composite films of cellulose nanocrystals and magnetite. Both the methods of sensing (dermal and oral) displayed peroxidase-like activity and the lower level of detection of glucose was 5 mM . The sensing was based on observing the color change (colorimetry) and hence can replace the amperometry technique used in glucometers. Moreover, the sensor exhibited better selectivity in an acidic environment and hence can be employed to monitor glucose levels through saliva and sweat instead of using blood for detecting biological glucose levels (Tracey et al. 2020).

Enzymatic biosensor for detecting lactose based on molecular organic framework of Co-hemin (Co-hemin MOF) and chitosan composite was developed by Choi et al. (2020). The biosensor demonstrated a broad linear detection range ($10\text{--}100 \text{ nM}$) and selectivity in the presence of similar interfering molecules (galactose, ascorbic acid, dopamine, glucose, and uric acid). The real-time detection of lactose in dairy products using the biosensor was compared with HPLC results and the results clearly proved the applicability of the study in the detection of lactose in commercially available dairy products.

The detection of Zidovudine (ZDV) used in the treatment of AIDS is necessary to study the adverse effects as well as the side effects caused by higher doses. Graphite electrode modified by a hybrid composite consisting of DNA, chitosan, graphene oxide, and bimetallic Au-Pt nanoparticles was reported by H.R. Akbari Hasanjani and K. Zarei (2021). The biosensor showed remarkable selectivity, a wider detection range ($0.01 \text{ pM}\text{--}10 \text{ nM}$), and a much lower LOD (0.03 pM). The real-time analysis of ZDV in human serum also produced reasonable results.

Electrospinning was employed to produce composite nanofibers of collagen, hemoglobin, and carbon nanotubes. The composite was further used for the electrochemical detection of hydrogen peroxide by amperometric and cyclic voltammetric methods. The higher surface area and porous nature of the nanofiber composites facilitated the direct transfer of electrons accelerating the electro catalysis and selective detection of H_2O_2 (Li et al. 2014).

The detection of physiological levels of dopamine using an apt sensor based on collagen (glass carp skin derived – GCSC) -graphene oxide composite system (GCSC-GO) was reported by Wei et al. (2019). The composite GCSC-GO was used as the transducer part and the biological recognition of dopamine was made possible using a label-free aptamer. The developed biosensor showed better selectivity, superior robustness, and feasibility during the amperometric determination of dopamine in human blood serum and hence can be utilized for dopamine sensing in clinical samples.

Immobilization of enzymes for optical sensing of biomolecules can be done using suitable matrix materials. Accordingly, hydrothermally grown titanium dioxide nanotubes (TNT-TiO₂) were incorporated in a polymeric matrix of alginate and then cross-linking using calcium ions (Ca^{2+} -cationic cross-linking) was done to get the nanocomposite hydrogel. The composite was initially screened for colorimetric sensing (blue color formation) of lactate and glucose in artificial sweat. The detection was very rapid in the case of TNT-alginate scaffold compared to bare alginate scaffold; 4 min and 6 min for lactate and glucose colorimetric sensing. The TNT-alginate scaffold was then impregnated on a cellulose paper strip and colorimetric detection of lactate and glucose was studied. The paper scaffold displayed homogeneity in sensing and a quicker response indicating the potential of the scaffold in devising wearable devices for real-time detection of glucose and lactate in sweat (Gunatilake et al. 2021).

A biosensor for the detection of histamine using a cryogel composite of chitosan and gold (Au) nanoparticles was reported by N. Nontipichet et al. Electrodeposition of Prussian blue (PB) was initially done on multiwalled carbon nanotubes coated on a carbon electrode (SPCE). The composite cryogel helped in the immobilization of the enzyme diamine oxidase (DAO). The method of preparation of the electrode and detection using amperometry is depicted in Fig. 1. The current generated during the reduction of PB during enzyme catalyzed oxidation of histamine was measured. The modified electrode showed two broad linear detection ranges with exceptional stability (operational and long-term storage), selectivity, and lower detection limit ($1.81 \mu\text{mol L}^{-1}$). The sensor showed exceptional recovery during the detection of histamine in shrimp and fish samples, and the results obtained using the biosensor were consistent with that of the results using the ELISA test (Nontipichet et al. 2021).

Chitosan was employed to increase the stability of carbon nanotubes in an aqueous solution to form a well-dispersed composite for the detection of the hormone leptin in blood serum. The single-walled carbon nanotubes (SWNTs) were functionalized using a cross-linking agent for 30 min in order to activate the functional group (carboxyl group) and dispersed in a chitosan matrix followed by the addition of leptin (with the non-specific sites of binding blocked using bovine serum

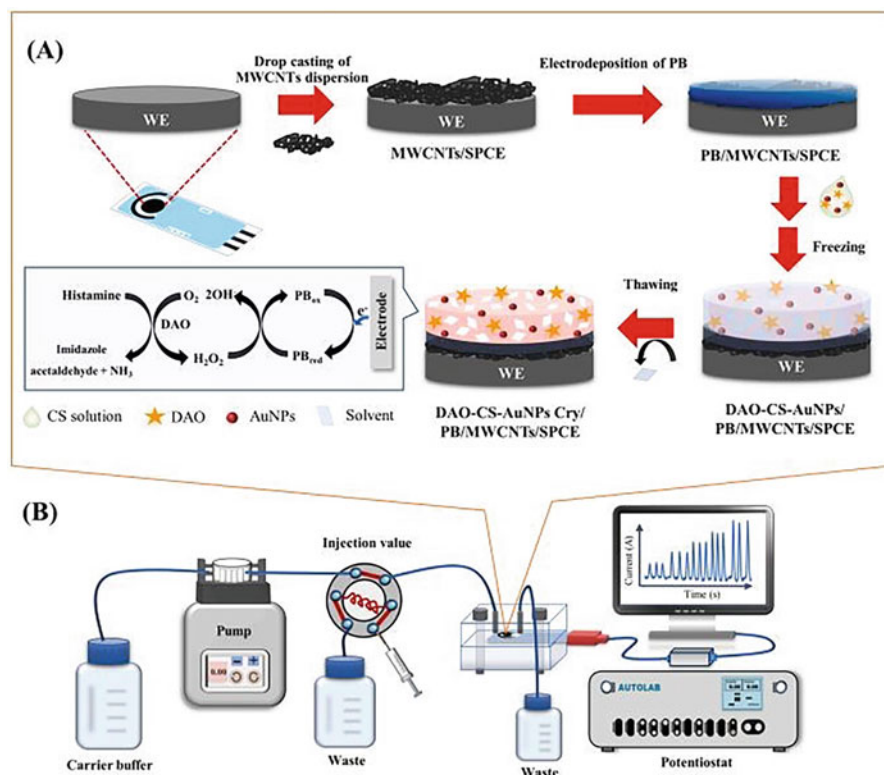


Fig. 1 Schematic representation of the preparation of modified composite electrode and the amperometric method employed for the detection of histamine

albumin) to obtain the biosensor. The sensor exhibited an appreciable recovery rate and the study can be extended for real-time detection of leptin in clinical samples (Zhang et al. 2018).

Miscellaneous Applications of Biopolymer-Based Nanocomposites

Biopolymers are used in developing hybrid composites with improved properties because of their biocompatible and biodegradable nature. In addition, the presence of multiple functional groups present in biopolymers helps in controlling the properties when combined with other nanomaterials. Biopolymer composites of cellulose, collagen, gelatin, proteins, alginate along with metal nanoparticles, metal oxides, carbon nanomaterials, etc., have received more consideration in the field of optics and electronics (Colusso and Martucci 2021).

Conducting a biopolymer composite film of collagen and copper nanoparticles has been reported by K. Cheirmadurai et al. The copper nanoparticles were prepared following a green procedure using henna leaf extract and discarded collagen was

utilized to develop the conducting film. The improved electrical conductivity of the films suggests the potential of the composite for various electronic applications. The film when introduced between batteries was capable of illuminating a LED lamp demonstrating its excellent electrical conductivity (Cheirmadurai et al. 2014).

Bacterial cellulose (BNC) possessing high purity has been used in combination with gold nanorods for developing Surface Enhanced Raman scattering (SERS) composite for the detection of bacteria, and lower concentrations of rhodamine have been reported by Tian et al. The porous and 3D structure of BNC helped in enhancing SERS making the composite beneficial for catalysis as well as energy storage applications (Tian et al. 2016).

Cellulose was employed as the template for developing a hierarchical nanocomposite consisting of phosphotungstic acid (HPW) and TiO_2 for photocatalysis applications. The composite was further evaluated as a photocatalyst for the degradation of methylene blue (Lin and Huang 2021). Another group reported the use of cellulose to properly disperse silver nanoparticles and zinc oxide nanoparticles for fabricating a composite for photocatalysis. The composite displayed better degradation of methyl orange (92% in 2 h) under sunlight as well as UV light (Shi et al. 2021).

A photocatalyst (TFCM) employing chitosan microspheres as a matrix for the effective dispersion of ferrite nanoparticles was reported by Nawaz et al. The degradation of methylene blue was investigated and the results obtained indicated the efficiency of TFCM as a photocatalyst in degrading 93% of the dye in 150 min (Nawaz et al. 2020). The relevant biopolymer-based nanocomposites reported for photocatalytic applications are given in Table 5.

Biopolymer-based supercapacitor electrodes have been reported because of their natural abundance, the easiest way of extraction, multifunctional nature, etc. (Pandiselvi and Thambidurai 2014). Cellulose and chitosan are the most exploited biopolymers for supercapacitor applications (Roy et al. 2021). They are either used in the form of composites with conductive materials or as a renewable source of carbon precursor.

Active electrode material based on chitosan and polyorthoamino phenol (POAP) for developing supercapacitors was reported by Ehsani et al. The electrode material

Table 5 Photocatalytic applications of biopolymer nanocomposites

Photocatalyst	Pollutant	Degradation efficiency and time	Reference
Chitosan loaded with Zr/ZnO	Hexavalent chromium	94.6% in 60 min	Preethi et al. (2020)
Carboxy methyl cellulose with Zinc ferrite (ZnFe_2O_4)	Ciprofloxacin	87% in 100 min	Malakootian et al. (2019)
Starch, bismuth tungstate, and graphene oxide	Ethylene	88.4% in 200 min	Xie et al. (2020)
Sodium alginate and TiO_2	Tartrazine	89% in 180 min	Dalponete et al. (2019)
Bacterial cellulose, SiO_2 , and TiO_2	Crystal violet	97% in 50 min	Rahman et al. (2021)

exhibited superior mechanical stability due to the incorporation of chitosan and demonstrated a higher value of specific capacitance (345 F/g at 100 mVs^{-1}) and retention of initial capacitance after 1000 cycles (Ehsani et al. 2019). Another study reported on chitosan and polyvinyl alcohol incorporated hydrogel electrodes with remarkable energy density (10.03 Wh kg^{-1}) and cycling stability (up to 5000 cycles) (Li et al. 2022). Cellulose aerogel and carbon composite were employed in developing an asymmetrical supercapacitor. The 3D porous hierarchical percolation of cellulose aided in electrolyte penetration, faster electron, and ion transport resulting in higher power (23 WL^{-1}), and energy densities (3.4 Wh L^{-1}) (Lei et al. 2021). Another group reported on cellulose nanofiber and graphene oxide fabricated as composite foam with improved electrochemical activity as an electrode for the supercapacitor. The foam electrode showed a higher specific capacitance (398.47 F/g at 0.5 A/g current density) and outstanding cycling stability even up to 10,000 cycles (Liu et al. 2021).

Future Outlook

Biopolymers have a significant role in various fields of applications owing to their good mechanical, thermal, and barrier properties. The development of biopolymers is necessary to reduce atmospheric pollution caused by synthetic polymers. But the cost of biopolymers is a factor that has to be addressed in further research. It is essential that biopolymers should be produced at a commercial level at a price comparable to synthetic polymers. The properties of biopolymers can be manipulated by the incorporation of various nanofillers in different compositions. In this respect, nanotechnology has a prominent role in improving the existing features of a biopolymer. The replacement of plastic materials that are used today has to be completely substituted with biodegradable polymers. Reusability of these plastics is not a final solution for the environmental issues. An ultimate solution will be possible only by the complete substitution of plastics for biopolymers. Such a process will assist in reducing pollution issues and leads to a sustainable future.

Conclusions

When environmental issues are concerned, recent research focuses on biopolymer-based nanocomposites for a variety of applications. However, many limitations in the processing and production of biopolymer-based nanocomposites have to be nullified in order to reach a commercial level. In this regard, a thorough study of natural biopolymers indicating the challenges faced in this area. This chapter summarizes the properties of biopolymers, the introduction of nanofillers to form bio nanocomposites, and their properties and applications in food packaging, drug delivery, wound healing, tissue engineering, and sensing. From recent studies, it is clear that blending of biopolymers and fabrication of nanocomposites are effective ways to get the desired results. The incorporation of nanofillers can optimize the

properties of the matrix along with the reinforcement provided. The chapter recommends novel, economic synthetic strategies of bio nanocomposites that can pave a new way in the field of polymers and can contribute new opportunities in various applications.

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Biopolymer-Based Blend Nanocomposites

20

P. K. Sandhya, M. S. Sreekala, and Sabu Thomas

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Abstract

Biopolymer-based blend nanocomposites can eliminate the adverse effects of plastic wastes and severe environmental problems. In recent years, researchers are attracted toward the development of new polymeric materials with desired properties without environmental impacts. The biodegradable blend nanocomposites can perform smart applications in industrial, pharmaceutical, and biomedical fields by tailoring its properties. The enhancement in properties of blends can be achieved by the addition of nanofillers with or without modifications. This chapter discusses the properties of biopolymer blend nanocomposites prepared from agro-resources and chemically synthesized using agro-based monomers in

P. K. Sandhya (✉) · M. S. Sreekala · S. Thomas
School of Chemical Sciences, Mahatma Gandhi University, Kottayam, India
e-mail: sabuthomas@mgmu.ac.in

detail. The applications and processing techniques of biopolymer blend nanocomposites are also summarized in this chapter.

Keywords

Biopolymers · Nanocomposite · Mechanical property · Thermal stability · Packaging application

Introduction

Biopolymers are a type of polymer that is derived from natural resources. Polymers can be derived from biological systems such as microorganisms, plants, crustacean shells, woods, mushrooms, and other organisms known as biopolymers. Cellulose, silk, lignin, hemicelluloses, and starch are some of the examples of biopolymers that occur in nature as macromolecules. Depending on the synthesis and sources biopolymers are classified into: (1) polymers from agro-resources (starch, cellulosic products, chitosan/chitin, etc.) and proteins and lipids from animals and plants (casein, whey, soya, and gluten), (2) polymers from microbial action (poly[hydroxybutyrate] [PHB] and poly[hydroxybutyrate cohydroxyvalerate] [PHBV]), and (3) chemically prepared polymers from agro-based monomers (polylactic acid [PLA]). Bio-based plastics are gaining popularity as a potential solution to problems caused by petroleum-based plastics.

The studies in the field of bio-based plastics are increased due to their similarities in the values of tensile strength and elastic modulus as that of synthetic polymers (D'Anna et al. 2022). However, brittleness and poor barrier properties limit their application in many fields and these limits can be overcome by blending with other biopolymers. Simple mixing of polymers without chemical reaction refers to blending and is the best way to create new materials with the desired properties. This technology can provide various advantages such as maximize material's performance, improvement in properties in relation to a specific application, decrease sensitivity to water, as well as application requirements at the lowest possible cost and in the shortest amount of time.

Blending is the frequently used method in the industrial sector because it is more practical and cost-effective. In some cases, the final properties of polymer blends can be done by modification of the blend using chemically modified polymers, or by mixing polymers with nanofillers at the time of processing to get good molecular interaction of the two polymeric phases present in the blend. For instance, PLA-PHB polymer blends are immiscible and their properties can be enhanced by modifying the blend with nanoclay (D'Anna et al. 2022). The nanofillers that are used in nanocomposites are divided on the basis of number of dimensions as fibers (1D), nanoplatelets (2D), and particles (3D) (Mochane et al. 2020). One-dimensional nanomaterials consist of nanorods, nanowires, and nanotubes. Two-dimensional nanomaterials exhibit plate-like shapes and nanolayers, nanocoatings and nanofilms are included in this category. Graphene is the well-known example for 2D material.

Three-dimensional nanomaterials may include nanoparticle dispersions, nanowire bundles, multilayers, and nanotubes.

The nanofillers may have an impact on the morphology of polymer blends by (a) changing the viscoelastic properties, (b) increasing component compatibility, and (c) changing physical barrier effect on the mixing of the blends (Vatansever et al. 2019). The localization of nanoparticles within the polymer blends has significant effect on the properties of the final product. The localization of nanoparticles in the polymer matrix leads to the breakup of the minor phase by immobilizing the polymer chains of the matrix. But, when the nanoparticles localize at the interface, it can act as a barrier between two phases and they can suppress the coalescence of the droplets. The morphology of the composites can be balanced with nanoparticles by reducing the interfacial interactions. When the nanoparticles located in the minor phase, nanoparticles can decrease the droplet size in the minor phase via “*cutting effect*” mechanism and it is observed when platelet-like nanoparticles such as nanoclays are used (Vatansever et al. 2019).

The thermodynamic factors originated from polar and the filler-polymer interactions control the localization of the filler in blend composites. It is observed from many studies that the filler has the tendency to locate in high-affinity polymer phase during mixing (Mochane et al. 2020). The filler concentrates at one phase in such a way that the interfacial energy gets reduced and filler prefers less viscous phase to minimize the interfacial energy. In addition to thermodynamic effect, kinetic effect plays a role in the localization of fillers in polymer blends. The kinetic effects like melt viscosity, shear rate, compounding sequence, and melt compounding time also affect the localization of the filler (Mochane et al. 2020). Melt viscosity has an important role in deciding the final morphology and final filler localization in polymer blends. Less-viscosity polymers possess better wetting coefficient and nanoparticles can easily diffuse into it. In compounding sequence, filler is premixed with less affinity phase and then mixed with second component, based on the concept of migrating filler toward more interacting phase from noninteracting phase. Different mixing times play a role in the localization of nanofillers in polymer blends and more mixing time is necessary for nanofillers to move from one phase to another.

Processing Techniques of Biopolymers-Based Blend Nanocomposites

Different methods are adopted for the synthesis of bio-based polymer blend nanocomposites. One of the methods for the preparation of biopolymer blend nanocomposites is solution casting method. Polyvinyl alcohol/starch/zinc oxide nanoparticle-based nanocomposite films were prepared using solution casting (Hu et al. 2022). Solution casting method of preparation of polyvinyl alcohol/starch/graphene oxide nanocomposites were reported elsewhere (Atta et al. 2021). Silver modified with hydroxyapatite nanoparticles AgHA-NPs/polyvinyl alcohol (PVA)/carboxymethyl cellulose (CMC) nanocomposites was also prepared by

solution casting technique (Nasrallah and Ibrahim 2022). Stearate Mg-Al layered double hydroxide (LDH)-based polyhydroxybutyrate (PHB)/polycaprolactone (PCL) blend nanocomposites were fabricated via solution casting intercalation method (Liau et al. 2014). Solution blending technique is used for PLA/starch/modified TiO₂/multifunctionalized graphene oxide nanocomposites (Wang et al. 2020). Melt blending is another method and used for the preparation of thermoplastic starch/poly(lactic acid) (TPS/PLA)/graphene blends (Solati et al. 2019). Melt blending is also used for fabrication of PCL/TPS/nanoclay blends (Guarás et al. 2016). Melt blending and compression moulding method is used for preparing thermoplastic starch (TPS)/poly(lactic acid) (PLA)/nanocellulose fibers composites (Nazrin et al. 2020). Melt mixing followed by hot pressing method is used for the preparation of cellulose nanocrystal/poly (lactic acid) (PLA)/poly (butylene succinate) (PBS) composites (Rasheed et al. 2021). Extrusion blowing method of preparation of starch/PBAT (poly[butylene adipate-co-terephthalate]) films containing silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnONPs) was studied (Zhai et al. 2022). PLA/PHB/clay blend nanocomposites were manufactured by twin-screw extrusion method (D'Anna et al. 2022). Twin-screw extruder and injection molding are other methods for the preparation of poly(ϵ -caprolactone)/starch/cellulose nanocrystals films (Manoel et al. 2022). Laser ablation technique is applied for the synthesis of carboxymethyl cellulose (CMC)/polyvinyl alcohol (PVA)/tungsten trioxide composites (Askary et al. 2022). PLA/PHB blends with GO and cinnamaldehyde were successfully achieved through a combined extrusion and impregnation process (Villegas et al. 2021). PLA/PHB/CNC composites were obtained using electrospinning method (Arrieta et al. 2016). PCL/gelatin/CNF-based nanocomposites were also manufactured via electrospun method (Moazzami Goudarzi et al. 2020).

Starch Blend Polymer Nanocomposites

Starch (ST) is a biodegradable and renewable natural biopolymer and it is one of the most important polysaccharides. It has been employed to develop environmentally friendly materials due to the rapid biodegradability and low cost. It consists of linear amylose with a spiral structure and highly branched amylopectin (Hu et al. 2022). The physical properties of starch are controlled by the ratio of amylose to amylopectin (Atta et al. 2021). The usage of starch in many applications, especially for packaging, is limited because of its great water sensitivity, low processability, and poor mechanical properties. These drawbacks of starch can be minimized by mixing starch with polyvinyl alcohol (PVA). PVA is a nontoxic and biodegradable material possessing high mechanical strength and high polar solubility in water. However, PVA has some drawbacks like high manufacturing cost, weak dimensional stability, and high water uptake. PVA and starch blends provide moisture-resistant, rapid biodegradable, low-cost, stable supporting medium with higher mechanical properties. The physical and functional features of PVA/ST blends can be improved by

adding various nanofillers like clay, graphene, cellulose nanocrystals, carbon nanotubes, halloysite, etc.

Hu et al. (2022) prepared polyvinyl alcohol/starch (PVA/ST) film filled with ZnO nanofillers (rod like) for food packaging. The stress-strain diagram for PVA/ST/ZnO nanocomposites is given in Fig. 1a–d. Tensile strength of virgin PVA/ST film is 15.6 MPa and elastic modulus around 114.7 MPa; for about 5 wt% of ZnO, the tensile strength of PVA/ST/ZnO composite was 22.9 MPa and elastic modulus was 182.6 MPa. About 46.8% rise in tensile strength and 59.2% rise in modulus of elasticity are observed as compared to neat PVA/ST composite. The nanocomposite films exhibit excellent water barrier performances, antibacterial activity against both *E. coli* and *S. aureus*, and outstanding UV-blocking ability. The nanocomposite films were found having application in active food packaging.

The physical properties of polyvinyl alcohol/starch blend composites can be improved by the incorporation of fillers such as graphene, biochar, and graphitic carbon nitride. Brittleness, flexibility, and processability of the composites can be improved by the addition of glycerol as a plasticizer in PVA/starch composites (Atta et al. 2021). The physical properties of polymers can be improved by adding graphene oxide (GO) as nanofiller, which consists of carbonyl, carboxyl, and

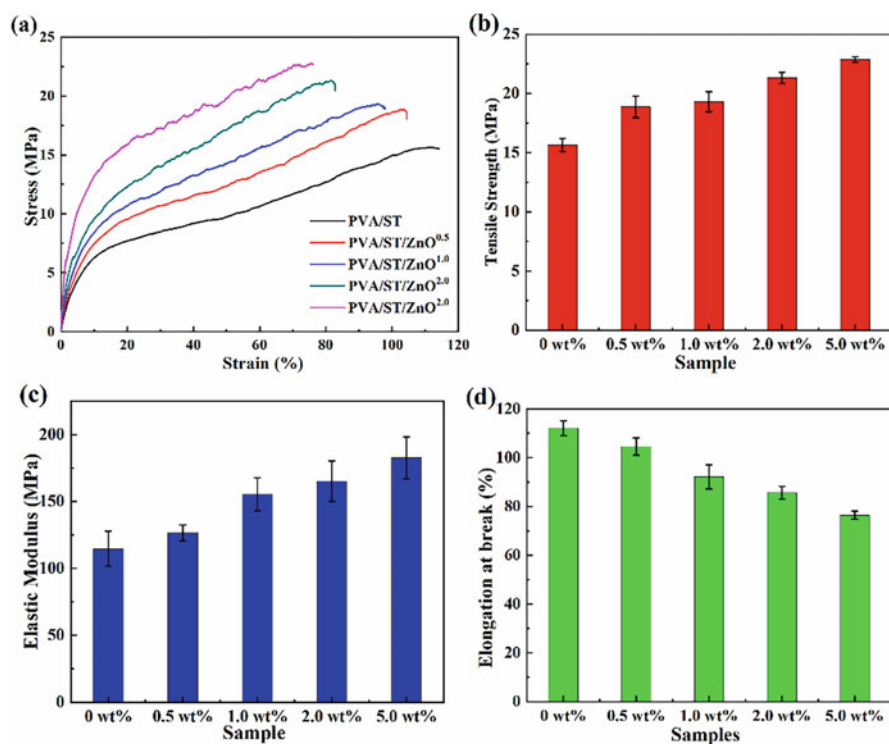


Fig. 1 (a) Stress–strain, (b) tensile strength, (c) elastic modulus, and (d) elongation at break for PVA/ST/ZnO nanocomposites (Hu et al. 2022)

epoxy functional groups. The presence of these functional groups helps in forming of strong bonds between GO and starch. On adding GO, an increase is observed in the tensile strength and moisture resistance of PVA/starch blend containing glycerol. Atta and coworkers (2021) successfully prepared PVA/starch/GO nanocomposite by solution casting technique and also studied the effect of various times of nitrogen plasma irradiation on nanocomposite. The plasma irradiation time has an influence on the thermal properties of nanocomposites and the results exhibited that the worst thermal stability of composites is observed after 10 min and best thermal stability is observed after 30 min. There is a decrease in storage modulus of unirradiated composites which is observed from 6.3 to 4.5 Mpa upon irradiation for 10 min and the storage modulus of nanocomposites enhanced to its maximum value after 30 min with further increasing exposure time.

Zhai and colleagues (2022) prepared starch/poly(butylene adipate co-terephthalate) PBAT films with silver nanoparticles (AgNPs) and zinc oxide nanoparticles (ZnONPs) by extrusion blowing method. They studied the physicochemical properties and antimicrobial activity of the films and found that the nanocomposite films containing silver and ZnO nanoparticles prevented the penetration of UV light. The addition of these nanoparticles increased the mechanical and barrier properties. SP-Ag(0.8)-ZnO (0.2) had the greatest inhibitory effect on *S. aureus* and *E. coli* because of the synergistic effect of silver and ZnO nanoparticles. Furthermore, initial packaging testing for nectarines and peaches revealed that the developed films possess excellent ability to prevent rot and maintain new product quality.

PLA-starch composites were prepared by Wang and coworkers (2020) using silanlated nano-TiO₂ (MTiO₂, 1% and 2%) and graphene oxide modified with maleic anhydride (f-GO, 0.1%, 0.2%, and 0.4%) at different combinations by solution blending technique. They investigated the collective effects of modified TiO₂/graphene oxide at various ratios on the structural characterization, morphology, thermal stability, crystallinity, and dynamic mechanical and antiaging properties. An obvious improvement in properties was observed in PLA-starch composites because of the better interfacial interaction and the collective effect from the combination of 1% MTiO₂ and 0.2% f-GO. The results showed that fabricated high-performance nanocomposites found application in food packaging. Solati et al. (2019) also studied the effect of graphene nanoplatelets on thermoplastic starch/poly(lactic acid) (TPS/PLA) blends and analyzed the compatibility, morphology, and crystallinity of the blend nanocomposites. SEM images were used to study the morphology of the blends and nanocomposites and found that the content of graphene sheets and blend composition determines the size of dispersed droplets (TPS). The DSC results showed that the addition of nanoparticles to the blend, cold crystallization temperature is decreased to zero and wetting coefficient values showed that the nanoplatelets were localized at the interface of PLA and TPS phases. From dynamic mechanical thermal analysis (DMTA), they found that the compatibility of PLA and TPS phases can be increased by adding about 1 wt% graphene nanoplatelets into the blend. Generally, the characteristic properties of thermoplastic starch/poly (lactic acid) blends can be improved by the addition of graphene nanoplatelets and are located at the interface

of the PLA and TPS phases. Also, the samples with graphene showed high percentage of crystallinity greater than TPS/PLA mixtures (Solati et al. 2019).

Manoel and colleagues (2022) extracted cellulose nanocrystals (CNCs) by acid hydrolysis and CNCs were incorporated into thermoplastic starch (TPS) phase mixed with poly(ϵ -caprolactone) (PCL) by higher shear rate corotating extrusion. About 3.25, 6.5, and 13 wt% CNCs were incorporated into TPS mass and only 3.25 wt% showed improvement in tensile strength by three times (12 MPa) and the elastic modulus by more than six times (1 GPa). The interactions between TPS molecules and CNCs with small concentration improved stability of the TPS/PCL nanocomposites ($\sim 250^\circ\text{C}$). Nanocellulose fibers obtained from sugar palm is used as reinforcement in TPS/PLA blend and investigated the thermal, mechanical, physical, and water barrier properties of nanocomposites from the group of Nazrin (2020). The morphology of the nanocomposites depends on TPS loading and the lower content leads to cracks and voids, while high content results in agglomeration. The prepared sample consists of 20%, 30%, 40%, 60%, and 80% TPS concentrations with constant palm nanocellulose fibers (0.5%). The Young's modulus of nanocomposites increased from 1.19–1.56 GPa up to 60% of TPS content and a decrease in the thermal stability is observed with increase in TPS loading.

The compatibility of hydrophilic starch with hydrophobic poly(lactic acid) (PLA) and poly(ϵ -caprolactone) (PCL) can be increased by adding polyhedral oligomeric silsesquioxane (POSS) nanoparticles. Oshani and coworkers (2019) studied the morphology, crystallinity, and thermal stability of nanocomposites and the results showed that 0.5 and 1 wt% POSS enhanced the compatibility of the system, while the higher contents of POSS resulted in the formation of aggregates. Transmission electron microscopy analysis showed that POSS is primarily localized within the PLA and PCL phases and PCL acts as an intermediate between PLA and starch. With a very low content of POSS of about 0.5 wt%, PLA's crystallinity increases from 22.9% to 31.6%, but the crystallinity of PCL is slightly hampered because of the formation of PLA crystallites. About 3 and 5 wt% of POSS increased the thermal stability of nanocomposites and good POSS dispersion within the matrix at lower contents is observed from the dynamic mechanical analysis.

A lot of research works were done in the field of PLA/TPS blend with different types of nanofillers. The addition of silica nanoparticles to PLA/TPS blend increased the elongation at break of the nanocomposites in the presence of maleated PLA (MPLA), which is hardened with polydimethylsiloxanol. Research works on nanoclays-incorporated PLA/TPS blends were reported and the presence of nanoclays improved the mechanical properties because of good adhesion between the two phases.

Cellulose-Based Blend Nanocomposites

Cellulose nanocrystals such as whiskers and nanospheres, nanofibers produced by electrospinning, cellulose nanofibrils, regenerated cellulose nanoparticles, etc. are the different cellulose-based nanomaterials. Cellulose nanowhiskers (BCNW)

obtained from bacteria is incorporated into gelatin-polyvinyl alcohol (GL/PVA) blend and analyzed the structural, mechanical, and water barrier properties (Haghighi et al. 2021). Needle-shaped BCNW with 7.5% content in GL/PVA blend showed decrease in water barrier properties and increase in mechanical properties. The developed blend composites were environmentally friendly and found application in packaging. Figure 2a shows the SEM images of the cross-section with compatibility and structural integrity between GL and PVA. A compact and repeated structure without air bubbles or pores with no phase separation is observed. Figure 2b–e showed the cross-section of films containing BCNW up to 7.5 wt% indicates homogeneous dispersion of BCNW and good miscibility of components. Figure 2f represents 10 wt% BCNW sponge-shaped and irregular structures because of poor interfacial bonding.

Polyvinyl alcohol (PVA) is a synthetic biopolymer which possesses significant tensile strength, nontoxicity, high crystallinity, and water solubility. PVA found applications in optical, biological, and electrical fields (Askary et al. 2022). Carboxymethyl cellulose (CMC) is a derivative of cellulose, nontoxic, biodegradable polymer. However, CMC possesses low chemical and physical properties and can be improved by blending. CMC/PVA blends nanocomposites with tungsten trioxide which were fabricated using laser ablation method and their optical, thermal, and electrical properties were studied (Askary et al. 2022). Thermal stability studies showed that nanocomposites exhibit higher stability with the addition of tungsten trioxide. Electrical property studies showed a decreasing trend in dielectric constant due to interaction between dipoles and applied field, but at high frequency constant values were obtained due to absence of interaction of dipoles.

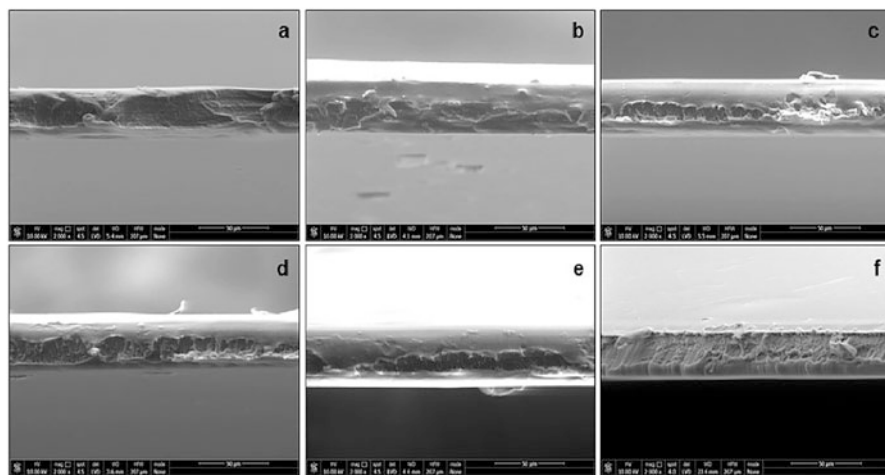


Fig. 2 SEM images of films based on (a) gelatin-polyvinyl alcohol blend (GL/PVA), (b) GL/PVA/bacterial cellulose nanowhiskers (BCNW) 1%, (c) GL/PVA/BCNW 2.5%, (d) GL/PVA/BCNW 5%, (e) GL/PVA/BCNW 7.5%, and (f) GL/PVA/BCNW 10% (Haghighi et al. 2021)

Silver-doped hydroxyapatite nanoparticles were incorporated into polyvinyl alcohol (PVA)/carboxymethyl cellulose (CMC) and investigated the morphology, optical properties, dielectric constant, and antibacterial activity of blends (Nasrallah and Ibrahim 2022). The results showed that the antimicrobial and electrical conductivity of the blend composites improved with the addition of nanoparticles and it is found that it has application in infection control and wound dressing. L-alanine amino acid-modified CuO nanorods were used as nanofiller in PVA/CMC matrix and the mechanical properties were investigated (Amaregouda et al. 2022). The studies showed that the resultant composites with 8 wt% CuO-L-alanine showed improved tensile strength and highly enhanced water vapors barrier and UV shielding ability. These nanocomposite films found application in food packaging. PVA/CMC matrix with graphene nanoplates (GNPs)/MWCNTs were prepared and examined the electrical properties along with structural and optical properties (Abutalib and Rajeh 2022). The highest AC conductivity of nanocomposite film was 4.53×10^{-4} S/cm (30 °C) and the improved optical properties made them good materials for electrochemical applications. Sharma and colleagues (2021) prepared TiO₂ sols and blended with cellulose/PVA film and SEM images showed that TiO₂ particles were distributed homogeneously throughout the film. The prepared films were thermally stable with enhanced photocatalytic activity by showing the degradation of 98.10% methylene blue under sunlight within 120 min. CNCs were modified using PVAc and reinforced in poly(L-lactic acid) (PLLA) matrix by industrial melt compounding method (Wu et al. 2022). CNCs-PVAc with 5 wt% in PLLA improved notched impact toughness by 57.0% and inter-layer adhesion strength by 103%.

Perumal et al. (2018) isolated cellulose nanocrystals (CNC) from agricultural waste rice straw (RS) that acts as reinforcement in polyvinyl alcohol/chitosan (PVA/CS) used for the preparation of bionanocomposite films. They found that the tensile strength (98.15 MPa) and thermal stability of the bionanocomposite films increased with increase in weight percentage of CNC (1–5 wt%). PVA/CS/CNC blend nanocomposite films show excellent antifungal property and it has applications in food packaging. The dispersion of cellulose nanocrystals and interfacial adhesion in PLA matrix is enhanced by grafting of CNC with glycidyl methacrylate (GMA) and an emulsion of CNC in poly(vinyl acetate) (PVAc) is prepared (Pracella et al. 2014). The morphology analysis showed better dispersion of CNC in all types of composites and high thermal stability is observed for CNCs dispersed in PVAc. PVAc dispersed CNCs composites with weight percentages less than 5 wt% showed high mechanical properties.

Cellulose nanocrystals (CNC) is usually obtained from sugarcane bagasse and possesses low density (1.566 g cm^{-3}), high elastic modulus (150 Gpa), and specific surface area, which makes them a good candidate for polymer reinforcement agent (Mofokeng and Luyt 2015a). Chitosan (CS) is a biopolymer, derivative of chitin and a linear polysaccharide, that found its applications in the field of pharmaceuticals, materials science, etc. (Miri et al. 2015) prepared the bionanocomposite films based on CNC/PVA/CS and studied the structural, morphological, mechanical, and thermal properties. They found that with the addition of 5 wt% CNC into PVA/CS blend improved the 105% increase in Young's modulus, 77% in tensile strength, and 68%

in toughness. Studies on cellulose nanostructures obtained from bacteria used as reinforcement in gelatin-polyvinyl alcohol (GL/PVA) blend were also reported (Haghighi et al. 2021). Miri and colleagues (2015) also developed biopolymer blend based on carboxymethyl cellulose (CMC) and starch (ST) using CNC as nanofiller. The resultant blend nanocomposites showed good optical transparency (80–95%) with 94.77% increase in elastic modulus. The increase in properties is attributed to the hydrogen bonding interactions between the functional groups of mixed components present in the blend. The developed blend composites found application in the field of packaging.

Pectin-Based Blend Nanocomposites

Pectin is a natural, nontoxic, water-soluble polysaccharide with β -(1–4)-D-galacturonic acid with arabinose, rhamnose, and galactose. It is present in plant cell walls. The presence of free carboxyl groups in pectin solutions shows acidic pH values. It is suitable for the synthesis of bio-based food packaging films because of its biodegradability, film-forming ability, gelling ability, and biocompatibility. It is used as additive in beverage and food industry, and colloidal stabilizer or thickener in ice creams, jams, milk drinks, etc. Its cytocompatibility helps to find applications in drug delivery, wound dressing, and tissue engineering (Priyadarshi et al. 2021).

Cavallaro and coworkers (2013) prepared pectin/polyethylene glycol 20,000 (PEG) films with halloysite nanotubes (HNTs) and studied its crystallinity, tensile properties, water uptake, tensile properties, and thermal stability. The factors that depend on wettability of developed films are distribution and composition of HNT and the surface roughness of the material. The results showed that nanoclays help to improve the tensile properties and reduced PEG crystallinity, and can be maintained by the addition of pectin. The developed film found application in packaging. Roy et al. (Roy and Rhim 2021) prepared pectin/agar composite films which were reinforced with melanin nanoparticles (MNP) and grapefruit seed extract (GSE). The presence of MNP improved the mechanical property of the film, whereas the inclusion of MNP and GSE increased strength of the film. The developed films exhibit excellent antibacterial activity against foodborne pathogens and antioxidant activity with the addition of MNP and GSE. Figure 3 represents the thermal stability analysis of pectin/agar-based films. The moisture in the film was evaporated at 50–110 °C and the second weight loss at 130–250 °C. The maximum decomposition occurred at 220 °C due to breakdown of glycerol and final weight loss occurred at 250–350 °C because of the decomposition of pectin and agar. The thermal stability of composite film is not much affected by the addition of fillers.

Saha and colleagues (2016) prepared pectin (PEC)/methylcellulose (MC) blended with sodium montmorillonite (MMT) nanocomposites films and studied the mechanical, thermal, and moisture absorption properties. Nanocomposites with 3 wt% MMT showed better mechanical properties and water vapor permeability, suitable for packaging applications. But 1–5 wt% MMT showed better thermal stability, and time of drug release suitable for drug delivery applications. The effects of TiO₂

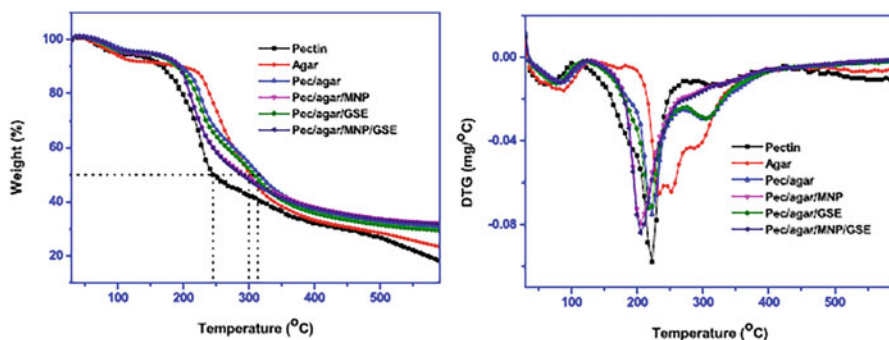


Fig. 3 (a) TGA and (b) DTG of pectin/agar-based bioactive composite films (Roy and Rhim 2021)

nanoparticles in starch-pectin edible films were studied and results showed that addition of low concentrations improved the moisture barrier and mechanical properties (Dash et al. 2019). The thermal stability of the composite films increased with increase in TiO_2 concentrations and the developed films found applications in food grade packaging.

Pectin and kappa-carrageenan biodegradable membranes were prepared with organically modified nanoclays using solution intercalation method and casting (Coelhoso et al. 2014). It is observed that about 10% of nanoclay decreased the water vapor permeability to 35% and 50% decrease in CO_2 permeability for 1% nanoclay; however, the increase in nanoclay content decreased the mechanical properties. Sodium alginate (SA) is an unbranched copolymer. In food, pharmaceutical, and medical fields it is used as film-forming agent and stabilizing thickener. SA/citrus pectin blends reinforced with nano- SiO_2 and the morphological, structural, and mechanical properties of the blend films were studied (Marangoni Júnior et al. 2022). The addition of 5% SiO_2 produced a significant improvement in barrier and mechanical properties (40.6 ± 4.5 Mpa) of films. The developed biopolymer nanocomposites were suitable for packaging. Biocomposites of hemicelluloses/pectin/nanocellulose were prepared and the effect of hemicellulose/pectin content from 0% to 100% with 25% nanocellulose on films was studied (Mugwagwa and Chimphango 2022). Pectin >50% biocomposites showed improved tensile strength and Young's modulus than hemicellulose-based biocomposites. The biopolymers exhibited slow release of antioxidant and are used for the packaging of fatty foods.

Gluten-Based Blend Nanocomposites

Gluten is the protein portion of the wheat. About 75–85% of dry weight of wheat is gluten and water insoluble. Gluten can be easily extracted from raw wheat, which consists of fat, carbohydrate, protein, and fiber. About more than hundreds of protein components are present in gluten.

Biodegradable nanocomposites films based on wheat gluten (WG), carboxymethyl cellulose (CMC), and cellulose nanofiber (CNF) were optimized using response surface methodology (RSM) (Bagheri et al. 2019). RSM method optimizes the response using various independent variables. It provides complete more accurate data with minimum number of experiments and optimizes the film formulation. The effect of WG, CMC, and CNF on color, water vapor permeability, and mechanical properties of the films were studied using RSM method. The experimental response values and predicted values were in good agreement with each other. The surface roughness of the film increases with CNF concentration and XRD results confirmed the amorphous nature of the films. Wheat gluten/carboxymethyl cellulose/cellulose nanofiber (WG/CMC/CNF) were prepared and investigated color and mechanical properties of nanocomposites using RSM (Bagheri et al. 2019). The results showed that there was good agreement between experimental response values and predicted values. The AFM results showed that surface roughness of samples increased with increase in the amount of CNF and XRD results confirmed the presence of amorphous nature of the films.

Baishya and colleagues (2018) prepared wood-starch-gluten nanocomposites and studied the morphological, mechanical, and thermal properties. Different ratios (70/30 and 50/50) of starch/wheat gluten blends were prepared and grafted with methylmethacrylate (MMA). To improve the properties of the blends modified TiO₂ nanoparticles were incorporated into the matrix and studied its effects on the properties of the composites. The morphological analysis showed that with 5 phr TiO₂ agglomeration of nanoparticles occurs and adversely affected the properties of the nanocomposites. Excellent mechanical properties as well as water and thermal resistance was observed for composites from 50/50 starch and gluten blend with 3 phr TiO₂.

Wheat-gluten/Cloisite 30B nanoclay-based composites were prepared by dispersing nanofiller into plasticized gluten via thermal processing (Zhang et al. 2007). The WG systems were strengthened by blending PVA with glyoxal cross-linking. The solid-state NMR study showed molecular-level distribution of polymer chain mobility and interactions between polymer and nanoparticles restricted the motion of all components in mobile phases. The nanoparticles had no significant influence in the mobility of the rigid phase. Wood starch gluten nanocomposites (WSGNC) were prepared by solution blending followed by compression molding and later TiO₂ nanoparticles were added to improve flammability and UV stability (Afnas et al. 2021). The results revealed that the presence of 3 phr TiO₂ in composites improved hardness and dimensional UV stability of the nanocomposites.

Gelatin-Based Blend Nanocomposites

Gelatin (GL) a mixture of polypeptide and protein, obtained from partial hydrolysis of collagen present in connective tissue, bone, and skin of various animals. It is abundant, low cost, and has no apparent color or flavor. GL films can prevent lipid and photo oxidation and act as good barrier to UV rays, light, and oxygen. These

properties improve the shelf life and quality of preservation of foods. However, poor water barrier as well as thermal and mechanical properties shorten the GL films food shelf life as compared to plastic films (Haghighi et al. 2021).

Afnas and coworkers (2021) successfully prepared PVA/gelatin/chitin blend and studied electrical, mechanical, water sorption, and antibacterial properties. The increase in chitin content produces an increase in electrical conductivity and possesses good antibacterial activity against gram-positive bacteria. The results of the study suggested that antimicrobial humidity sensors can be developed from blend nanocomposites. Roy et al. (Roy and Rhim 2022) developed sulfur nanoparticles (SNP) and incorporated them into gelatin/CNF composite films and used enoki mushroom extract for stabilizing SNP. The fabricated films exhibited UV protection and about 25% improvement in tensile strength. It also exhibited strong antibacterial activity toward bacteria and applicable for food packaging. The time-dependent antimicrobial activity of films toward *E. coli* and *L. monocytogenes* showed that there is no antibacterial activity observed for neat gelatin/CNF films; however, SNP filled films showed antibacterial action against both bacteria. The higher antibacterial activity of green SNP-based nanocomposite derived toward *L. monocytogenes* is due to the variation in cell wall construction and permeability.

A smart/active packaging materials based on gelatin/ κ -carrageenan with TiO_2 and anthocyanins were prepared by casting method (Sani et al. 2022). The moisture resistance as well as optical and mechanical properties of the composites were enhanced by TiO_2 and anthocyanins. The developed films were capable to inhibit oxidative reactions and great bacteriostatic properties. These biodegradable films can replace plastic packaging materials.

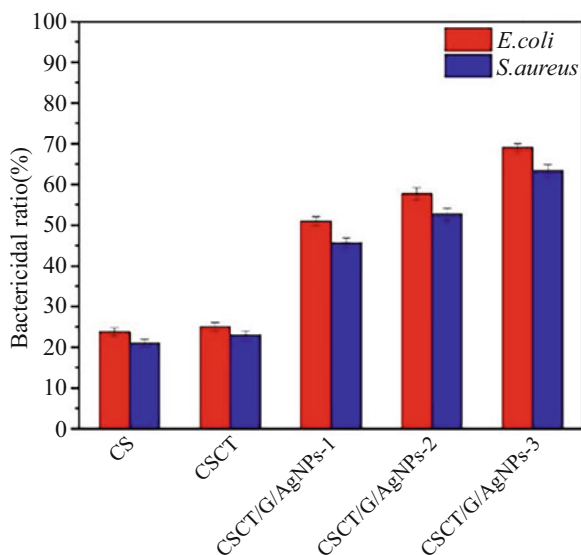
Gelatin/polyvinyl alcohol (GL/PVA) blended with bacterial cellulose nanowhiskers (BCNW) and the effect of BCNW on morphological, mechanical, and water barrier properties of the film was analyzed (Haghighi et al. 2021). Morphological results showed that with about 7.5 wt% BCNW there was good miscibility between GL/PVA and nanofiller. The concentration of BCNW up to 7.5% reduced water vapor permeability and increased the tensile strength (21.5%) and elastic modulus (19%). The GL/PVA/BCNW film was found useful in environmentally friendly packaging applications. Cellulose nanocrystals separated from water hyacinth fiber (WHF) were used for the preparation of PVA/gelatine/CNC composite films (Oyeoka et al. 2021). The addition of CNC improved the thermal stability and 5 wt% CNC provides maximum value of storage modulus (3 GPa). CNC incorporation decreased the water vapor permeability (WVP), moisture uptake, and water absorption. PVA/gelatine/CNC nanocomposites found application in food wrappers.

Gelatin-chitosan/MMT nanocomposites were prepared by electrospinning and morphology of the developed nanocomposites was studied (Habibi et al. 2019). The exfoliation of MMT layers in gelatin-chitosan matrix is observed from the XRD result and nanocomposites can act as great candidate in biomedical application. Poly (caprolactone) (PCL)/gelatin (GL) composites were blended with cellulose nanofibers (CNF) by electrospun (Moazzami Goudarzi et al. 2020). Different concentrations of CNF were incorporated into different PCL/GL ratio and studies showed that

the highest tensile strength is observed for P70/2CNF and maximum modulus is associated with P30/2CNF. GL blending and electrospinning decreased the crystallinity of PCL but the addition of CNF increased PCL/GL blends crystallinity. Cao and coworkers (2020) used catechol-modified chitosan (CSCT) for the reduction of Ag NPs and prepared CSCT/gelatin (G)/Ag NP film. The studies showed that the water vapor resistance and tensile strength of the composites increased by the presence of Ag NPs and G. The antibacterial activities of the chitosan (CS), CSCT, and CSCT/G/Ag NPs membranes against *S. aureus* and *E. coli* were shown in Fig. 4. The bactericidal rates of CS membrane against *S. aureus* and *E. coli* are 20.5% and 23.7%, respectively, because of electrostatic interaction between the negative charge present on microbial cells and positively charged CS. The presence of positively charged amino groups on CSCT causes antimicrobial activity. CSCT-Ag NPs showed higher bactericidal ratio which is due to greater antibacterial activity of the silver nanoparticles. The superior antibacterial activity of silver nanoparticles against gram-negative bacteria is because of decrease in the anchoring sites on the bacterial cell wall as a result of rigid structure of polysaccharide chain.

Studies on chitosan/gelatin films with tannic acid/bacterial nanocellulose were reported and are applicable for wound healing purpose (Taheri et al. 2020). Aldehyde-modified carrageenan/gelatin/halloysite nanotubes (AD-Carr/Gel/HNTs) nontoxic composites were prepared and act as a good candidate for tissue engineering purposes (Akrami-Hasan-Kohal et al. 2020). Low cost, biologically safe PVA/gelatin/0.01 CuO nanocomposites can absorb various UV subtypes and used for UV shielding applications (Ebnalwaled and Ismaiel 2019). These nanocomposites also found applications in γ -ray sensors and optical communications.

Fig. 4 Bactericidal ratios of CS, CSCT, and CSCT/G/Ag NP films (Cao et al. 2020)



Soy Protein–Based Blend Nanocomposites

Soy protein isolate (SPI), a by-product obtained from soybean oil industry, is abundant, low cost, and possesses film-forming property. It consists of 90% pure protein and tough substance. However, SPI films possess some drawbacks such as high water sensitivity, low mechanical strength, and poor thermal and barrier properties. Due to these drawbacks its application is limited in packaging industry (Xiao et al. 2020).

Sasmal and coworkers (2009) prepared maleated polycaprolactone/soy protein/clay-based eco-friendly bionanocomposites and studied the tensile and dynamic mechanical properties. Tensile strength and storage modulus of the nanocomposites are higher for clay-based nanocomposites. The biodegradability studies of the nanocomposites showed that increase clay content decreases the degradation time. The water absorption rate of nanocomposites with a rapid increase is observed in first 50 h and reaches equilibrium. A decrease in equilibrium water uptake and diffusion coefficient is due to (1) better interaction between groups on MMT nanolayer and amide groups on SPI and (2) large number of physical cross-linking formed by strong hydrogen bonding, limit swelling, and low water absorption.

Polycaprolactone (PCL)/soy protein isolate (SPI)/organoclay composites were prepared and morphology studies revealed that silicate layers were homogeneously dispersed at nanometer range (Nayak et al. 2008). The dynamic mechanical and tensile properties of the nanocomposites showed remarkable enhancement because of stiffening effect of nanoclay. Montmorillonite (MMT) was used as nanofiller in soy protein isolate (SPI)/polyvinyl alcohol (PVA) blends and the results concluded that MMT content had significant effect on the structure of composites (Guo et al. 2019). Due to the better reinforcing effect between MMT and polymers Young's modulus, tensile strength of the composites increased with rise in MMT content. Thermal stability of the composites improved, while the water sensitivity decreased because of the restriction of MMT lamellae on polymer segment. The developed nanocomposite found application as green packaging films.

Mansoori and colleagues (2019) prepared corn starch (CS)/soy protein isolate (SPI) blended with graphene (G) and graphene oxide (GO). They investigated the effects of G and GO in mechanical and physical properties of nanocomposite films. The modulus of 0.5 wt% G showed 578 Mpa while 2 wt% GO showed 449 Mpa. CS/SPI/GO (2 wt %) are thermally stable than pure starch/protein composite and exhibits lowest oxygen permeation rate. The addition of polyvinyl alcohol (PVA), 1,2,3-propanetriol-diglycidyl-ether (PTGE), and halloysite nanotubes (HNTs) into SPI matrix resulted in the formation of nanocomposite films (Liu et al. 2017). About 329.3% increase in tensile strength and 5.1% decrease in water absorption properties is observed. The green and transparent film can replace nonrenewable films in packaging.

Poly-Lactic Acid (PLA) Blend Polymer Nanocomposites

Polylactic acid (PLA) is a biopolymer derived from plants and it can be prepared synthetically by direct polycondensation and ring-opening polymerization (ROP) of lactide. PLA is biocompatible and biodegradable that degrades into water and carbon

dioxide but the properties like poor processability, slow crystallization rate, and vulnerable to aging have limited its applications in food packaging. The low degradation rate of PLA can be overcome by blending it with other biopolymers (with higher degradation rates).

D'Anna and colleagues (2022) studied the influence of process parameters on the microstructure and properties of PLA/PHB/clay blend nanocomposites. The crystallinity content of PLA (+25%) is increased by the nucleating action of clay nanofillers that are localized at the interface between PLA and PHB. They found that the thermomechanical properties of clay-filled blends processed through screw profiles 1 and 3 show 20% improvement in modulus due to the reinforcing effect of the clay. While in the case of blend processed through screw profile 2 only 9% increase in modulus is observed. The developed PLA/PHB/clay blend nanocomposites found potential applications in rigid packaging, personal care and cosmetic products, and toys.

Multifunctionalized graphene oxide was added to poly(lactide)-starch matrix by Wang et al. (2019) using solution casting method. For this study they used graphene oxide (GO) grafted with maleic anhydride and subsequently modified by dodecyl amine and analyzed morphology, crystallization property, dynamic mechanical properties, aging resistance, and surface wettability of the nanocomposite. The prepared f-GO-reinforced nanocomposites showed significant improvements in thermal stability, storage modulus, UV shielding capacity, surface hydrophobicity, and aging resistance properties.

The effect of TiO_2 nanoparticles on melt-mixed poly(lactic acid) (PLA)/poly(ϵ -caprolactone) (PCL) blend nanocomposites was studied by Mofokeng and coworkers (Mofokeng and Luyt 2015b). They give importance to the morphology and thermal stability analysis of blend nanocomposites. The morphology studies showed that the nanoparticles were localized in the PLA phase of the PLA/PCL blends, because of the larger affinity between PLA and TiO_2 due to slight difference in relative polarities between them. TGA analysis showed that the thermal stabilities of both polymers in the blends increased due to the presence of TiO_2 nanoparticles. The titania nanoparticles also affect the evaporation of degradation products from the blend by acting as degradation catalyst or prevent the escape of volatile degradation products.

Poly(lactic acid)/chitosan/functionalized graphene nanoplatelet PLA/CS/FGNP blend nanocomposites were successfully prepared by Paydayesh et al. (2022). The morphological, mechanical, and electrical conductivity and biodegradability of blend nanocomposites were investigated. The finest morphology of blend nanocomposites is observed with 3 phr FGNP (PLA75/CS25/FGNP3) with narrowest size distribution. Blend nanocomposites with 3 phr FGNP shows approximately 142% increase in tensile strength and 261% increase in Young's modulus with improvement in thermal stability. Moreover, in the case of electrical conductivity, there is a good agreement between the experimental data and model predictions. The electrical conductivity of PLA/CS/FGNP blend increases from 10^{-12} to 2.42 S/cm and threshold value was 0.48 vol%.

The cellulose nanocrystals (CNC) obtained from bamboo fiber can be used as filler in (PLA)/poly (butylene succinate) (PBS) composites. Rasheed and colleagues (2021) studied the physiochemical, morphological, thermal, and crystalline properties and tensile properties of the composites prepared via melt mixing followed by hot pressing. The morphological results showed that rod-like CNC particles are embedded in the homogeneous PLA-PBS blend and 1 wt% of CNC exhibited top value of tensile strength, tensile modulus, and thermal stability. PLA-PBS/CNC polymer blend found applications in packaging. The toughness of PLA can be boosted with epoxidized natural rubber (ENR) and nano-SiO₂ (Boonmahitthisud et al. 2021). In this work, 30 mol% epoxidation of ENR was synthesized and melt-mixed with PLA by changing weight percentages of ENR. The 80/20 PLA/ENR-30 composite showed better mechanical properties and selected for the preparation of SiO₂-filled nanocomposites. The distribution of SiO₂ is observed in the ENR-30 phase and not in PLA phase with improved thermal stability and decrease in mechanical property.

Polyhydroxybutyrate (PHB) Blend Polymer Nanocomposites

Polyhydroxybutyrate (PHB) is biocompatible, nontoxic, and hydrophobic material which can be synthesized biologically or chemically. PHB-based composites found applications in various fields like agriculture, health, bioremediation, and food packaging (Moreira et al. 2022). Tensile yield strength of PHB is found to be <10 MPa and the elongation at break is observed to be ~5%. Due to these low-value commercial applications of PHB, it is limited and can be improved by adding a variety of additives.

Cinnamaldehyde (Ci) was incorporated into PLA/PHB blends PLA/PHB/GO bionanocomposites using supercritical CO₂ and results showed that GO maintains thermal stability of PLA film while PHB decreases degradation temperature of PLA (Villegas et al. 2021). More flexible materials with 16% of increase in elongation percentage were observed and the bionanocomposites found application in drug delivery platforms. Poly(propylene carbonate) (PPC)/PHB bionanocomposites were blended with CNC 18 times and increase in oxygen barrier properties increases with 1 wt% CNC. The developed PPC/PHB/CNC nanocomposites can be used as degradable material for food packaging (Jiang et al. 2020).

PHB/thermoplastic starch (TPS) bionanocomposites were reinforced with montmorillonite clay using melt-blending and the mechanical and thermal properties were investigated (Garrido-Miranda et al. 2017). The studies showed that degree of crystallinity decreased with increase in amount of clay without significant change in thermal properties. About 22% increase in elastic modulus was observed when the amount of organically modified montmorillonite (OMMT) is 5%. The effect of Ag and Cu nanoparticles on properties of PLA and PHB blend were reported and resultant nanocomposites showed significant improvement in tensile properties (Cárdenas-Triviño et al. 2019).

TGA tests of PLA/PHB and PLA/PHB/nanoparticle (NP) mixtures show two decomposition temperatures except PLA/PHB in 70/30 ratio due to miscibility. PHB/PCL blends reinforced with Fe_3O_4 and CNFs and the mechanical properties and shape-memory performance of 3D-printed products were investigated (Yue et al. 2021a). The addition of Fe_3O_4 causes an increase in tensile strength of PHB/PCL blend while 10 wt% Fe_3O_4 and 0.5 wt% CNFs affected the strength and toughness of PHB/PCL blend. The optimum mechanical and shape-memory properties were exhibited by PHB/PCL blends in the ratio 80:20 with 0.5 wt% CNFs and 10 wt% Fe_3O_4 . The key thermodynamic parameters help in shaping memory behavior and measuring storage modulus and glass transition temperature (T_g). Figure 5 represents the storage modulus and $\tan \delta$ with temperature and E' curve that shows three areas, the glassy state ($>45^\circ\text{C}$), glass transition state ($45\text{--}65^\circ\text{C}$), and the rubbery state (above 65°C). A high value of E' is observed in PHB/PCL matrix when there is an increase in Fe_3O_4 content. In composites with Fe_3O_4 , JF20% shows E' value of 6436 MPa, higher than composites without Fe_3O_4 , because of increase in interaction between Fe_3O_4 and matrix. Figure 5c shows addition of rigid CNFs has little effect on E' of JF10% sample due to decrease in molecular chain entanglement. Fe_3O_4 increases the T_g because of the strong interaction between Fe_3O_4 and the matrix

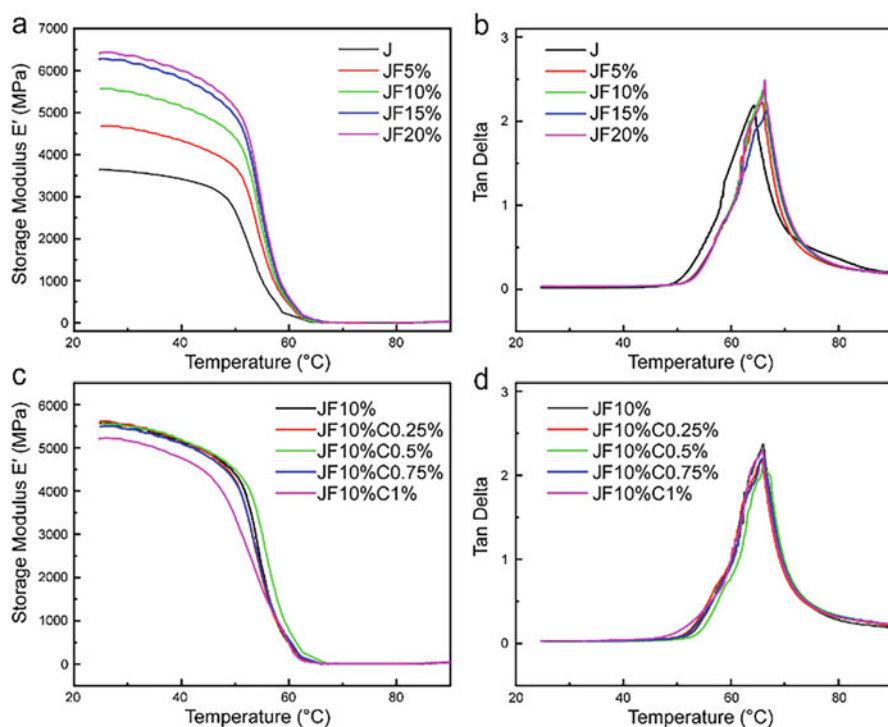


Fig. 5 E' (a and c) and $\tan \delta$ (b and d) of composites with different Fe_3O_4 and CNFs contents (Yue et al. 2021a)

(Fig. 5b) and CNFs have little effect on the T_g with 10 wt% Fe_3O_4 because small amount of CNFs has no influence in polymer chain mobility.

Yue and coworkers (2021b) also studied the effect of CNFs in PHB/PCL composites and found that 1 wt% CNF in 80:20 PHB/PCL composites exhibited the excellent mechanical and shape-memory properties. The synthesized PHB80/PCL20/CNFs nanocomposites found applications in bone tissue implants, smart robotics, four-dimensional (4D) printing, and expandable structures. The PLA/PHB/CNF blend improves the mechanical properties of PHB due to good interaction between CNF and polymers (Aydemir and Gardner 2020). DSC results showed that PLA improved thermal stability but CNF does not produce change in thermal stability of the composites. The mechanical properties of PHB can be improved by PLA and CNCs from sugarcane with a green plasticizer, epoxidized canola oil (eCO) (Lopera-Valle et al. 2019). The blending of PHB and PLA was improved by adding zinc acetate. The results showed that PLA (25 wt%) and CNCs at low concentration improved the Young's modulus while eCO (10 wt%) leads to an increase in strain at break.

Arrieta and coworkers (2014a) prepared nanocomposites PLA/PHB blends with CNC or surfactant-modified CNCs and found that CNCs improved the thermal stability, Young modulus, and elongation at break. The prepared nanocomposites opened a new perspective for food packaging application for short-term period. In another work reported by Arrieta (Arrieta et al. 2014b), surfactant-modified CNC for the preparation of nanocomposites with PLA/PHB blends was used and the morphology and thermal stability were investigated. The nucleation effect of PHB increased the crystallinity of PLA and well-dispersed nanofillers increased the thermal stability and interaction between PLA and PHB. Studies based on 15 wt% of acetyl(tributylcitrate) (ATBC) plasticized PLA-based PLA-PHB-ATBC with CNC or surfactant-modified CNCs bionanocomposite films were also reported from the group of Arrieta (Arrieta et al. 2015). Better results were observed for composites with modified CNCs due to synergic effect of plasticizer and nanofiller. In another work from (Arrieta et al. 2016), biodegradable nanocomposites were developed by electrospinning PLA with PHB and plasticized with 15 wt% ATBC and reinforced with CNC. They studied the morphological, mechanical, and thermal properties of the electrospun bionanocomposites. The results showed that ATBC decreased T_g and improved elongation at break, and CNC enhanced the thermal and mechanical properties with 1 wt% of CNC-filled bionanocomposites.

Vermiculite (VMT) is a type of silicate used for nanocomposite production and is hydrophilic in nature. Blends based on PHB/PP-g-MA (polypropylene grafted with maleic anhydride) and PHB/PP-g-MA/vermiculite nanocomposites were prepared by melt intercalation method and then compression molded (de Mesquita et al. 2016). The thermal behavior of blends and bionanocomposites showed that onset degradation temperature and T_m (melting temperature) get decreased. The decrease is high for bionanocomposites due to the interactions between clay and the maleic anhydride groups. The interactions helpful for biodegradation makes the system more amorphous and favors microbial attack.

Polycaprolactone Blend Nanocomposites

Polyhedral oligomeric silsesquioxane functionalized with glycidylisobutyl (G-POSS) is used as a filler in polycaprolactone/polypropylene carbonate ((PCL/PPC) blends and the shape memory properties along with morphological and mechanical properties were investigated (Kamyab et al. 2021). Scanning electron microscopy results of blends showed droplet-matrix morphology and it changed to co-continuous one with the addition of nanoparticles. The nanoparticle content and polymers mixing ratio affect the shape memory properties and optimum properties were observed for 20/80 PCL/PPC ratio with 5 wt% G-POSS. Studies on the effect of POSS on thermal stability and crystallization of PLA/PCL blends were reported (Moeinifar et al. 2020). The crystallization of blends was studied using differential scanning calorimetry (DSC) and the results showed that the solution crystallization of PLA is suppressed at low concentrations of POSS. The decrease is due to the better interaction between PLA and PCL. A higher degree of crystallinity is observed at concentration of POSS because of the nucleating effect of the nanoparticles. From TGA studies, it is found that the increase in thermal stability is observed for composites with low POSS content. The high content of POSS decreased the thermal stability and the reason for the increase or decrease in thermal stability is the decreased compatibility between PLA and POSS.

Layered silicate/starch polycaprolactone blends were prepared using three different clays and the mechanical properties of the composites were studied (Pérez et al. 2007). X-ray diffraction studies showed that clays were intercalated within the matrix and mechanical properties improved with increase in clay content. The greatest compatibility of Cloisite 10A with the matrix makes them to achieve best properties among other clay-based composites. They have also studied the water uptake behavior of composites and the results revealed that water absorption decreases with increase in content of clay (Pérez et al. 2008). The decrease in mean free path of water molecule leads to decrease in water absorption.

PHB/PCL/stearate Mg-Al layered double hydroxide (LDH) blends were prepared by Liao and colleagues (2014) and the structural, morphological, and mechanical properties were analyzed. XRD analysis showed that low filler concentration results in exfoliated nanocomposites. Transmission electron microscopy (TEM) images indicated the homogeneous distribution of LDH layers in the PHB/PCL blend. With 1 wt% nanofiller filled blends showed 66% enhancement in tensile strength than PHB/PCL blends. PCL/TPS (thermoplastic starch) and PCL/PCL-g-MA (anhydride-functional polycaprolactone)/TPS blends reinforced with natural montmorillonite and organomodified montmorillonite was fabricated by Guarás et al. (2016). XRD results showed the intercalated structure of nanoclays in the matrices and modified clays showed higher dispersion. There was no significant change in the thermal stability of pure PCL and TPS with addition of clay or TPS/PCL compatibilization. The Young's modulus increased with the addition of nanoclays, while a decrease is observed in the water adsorption of the compatibilized matrix.

Vinyl functionalized graphene (VGN) acts as an efficient compatibilizer for PLA/PCL blends that resulted in mechanically strong nanocomposites (Wang et al.

2022). It was observed that with 0.5, 1.0, and 2.0 wt% of VGN, a decrease in the phase size of co-continuous PLA/PCL blends with increase in tensile strength by 200%, 280%, and 253%, respectively.

Tubular bionanocomposite blends of PCL/PGS filled with nanoclay or GO (graphene oxide) were prepared successfully and the dynamic morphological, mechanical, and thermal properties (Davoodi et al. 2022) were studied. FESEM results revealed the droplet-matrix morphology throughout PCL/PGS blend and nanoparticles in ordered porous zones in PCL/PGS blend. Addition of GO increases the storage modulus and Tg from -25°C to -17°C of the PCL/PGS blend.

Polyhydroxybutyrate Valerate Blend Nanocomposites

Poly(hydroxybutyrate-co-valerate)/poly(ϵ -caprolactone) (PHBV/PCL) blends filled with TiO_2 nanoparticles were prepared and the morphology and thermal stability were investigated (Mofokeng and Luyt 2015c). The morphology analysis showed that the nanoparticles were dispersed well in PHBV/PCL blend with co-continuous morphology. Thermally stable PCL improved the thermal stability of PHBV but PHBV makes decrease the thermal stability of PCL. The improvement in thermal stabilities of PHBV and PCL in blend was observed only with 1 wt% of TiO_2 . Another work reported in PLA/PHBV/ TiO_2 nanocomposites analyzed the morphology, mechanical properties, and crystallization behavior (Gasmi et al. 2019). The increase in PLA content showed some uncommon morphology such as isothermal crystallization, which complicated the crystallization kinetics of the samples. The tensile properties of nanocomposite depend on blend and nanocomposite composition, which in turn relates to morphology. Zhao and coworkers (2013) prepared PLA/PHBV blends and PLA/PHBV/clay nanocomposites. PLA/PHBV/clay nanocomposites exhibited high melting strength and modulus than PLA/PHBV and nanoclay raises the cell density and decreased the average cell size of microcellular blend. The crystallinity of PHBV is improved due to the nucleating action of nanoclay. The addition of nanoclay improved Young's modulus of blends, but there is no improvement for the tensile strength.

Chitin nanowhisker filled PLA and PLA/PHBV blended nanocomposites and the corresponding nanocomposite foams were prepared by Guan et al. (Guan and Naguib 2014). They developed solid nanocomposites by melt blending and porous nanocomposite foams by batch foaming process using CO_2 as blowing agent. The results revealed that chitin nanowhiskers less than 2 wt% improved the mechanical properties of solid nanocomposites. PHBV/PBS blends with different ratios (80/20, 50/50, and 20/80 w/w) were reinforced with halloysite nanotubes (HNT) and the morphological and thermal properties of the blend nanocomposites were studied (Kennouche et al. 2016). Morphological studies showed that the dispersion of PBS in PHBV/PBS 80/20 blends was improved due to the diffusion and emulsifying effect of maleic anhydride-grafted PHBV. Addition of HNT in the blends improved fire reaction, decreased thermal stability, but the presence of PHBV-g-MA balances the two effects.

Applications of Biopolymers-Based Blend Nanocomposites

With the growing consumer concerns about the environmental impact of modern food packaging system, there has been great importance given to environmentally friendly packaging materials. Starch-based biopolymer blend nanocomposites with different nanofillers like ZnO, silver nanoparticle, TiO₂, etc. were used as packaging materials (Hu et al. 2022; Wang et al. 2020; Zhai et al. 2022). PVA/elatine/chitin ternary blend composites were identified for developing humidity-sensing user-friendly materials (Afnas et al. 2021). Anthocyanin-loaded gelatin/κ-carrageenan films reinforced with TiO₂ nanoparticles found application as smart and active packaging materials (Sani et al. 2022). Gelatin/polyvinyl alcohol filled with bacterial cellulose nanowhiskers was also used for food packaging applications (Haghighi et al. 2021). Chitosan/gelatin/tannic acid/bacterial nanocellulose films found application in wound healing purpose (Taheri et al. 2020). Carrageenan/gelatin/halloysite-based nanocomposite is used for tissue engineering applications (Akrami-Hasan-Kohal et al. 2020). Soy protein isolate-based nanocomposite containing cellulose nanocrystals and zinc oxide nanoparticles films is used for pork preservation (Xiao et al. 2020). PLA-PHB blends filled with functionalized CNCs can be used as short-term food packaging in industry (Arrieta et al. 2014b). Functionalized graphene oxide/poly(lactide)/starch nanocomposites pave the way for the development of nanohybrids in food packaging and pharmaceutical industries (Wang et al. 2019). Nanocomposite films based on PLA/PHB/cellulose nanocrystal are best for food packaging industry (Arrieta et al. 2014b). Composites based on polylactide/thermoplastic starch/nanosilica were found as good for applications in electronics materials, tissue engineering, disposable goods, surgical sutures, and drug delivery. Aldehyde-modified kappacarrageenan/gelatin blend modified with halloysite nanotubes act as good candidate for biomedical applications (Akrami-Hasan-Kohal et al. 2020).

Conclusion

The studies on the properties of biobased blend nanocomposites for the development of novel materials with specialized properties depend on the nature of nanofillers and method of synthesis. The incorporation of functionalized nanofiller into biopolymer blends decreases the agglomeration and water absorption properties while the mechanical, thermal, and electrical properties of the blends increase. The improvement in properties is due to the interfacial interactions of the groups such as carboxyl and hydroxyl present in the polymer matrix with nanofiller. The selection process of the combinations of biopolymer blends and nanofillers for the synthesis composites followed without a well-established scientific principle and as a result some composites exhibit excellent physicochemical properties and reinforcement effects. On considering the cost, availability, processing methods, and applications in various fields, there have been more chances for success in commercialization of biopolymer-based blend nanocomposites. More research is essential for utilizing various nanoparticles for producing new blend nanocomposites with improved properties.

Cross-References

- [Biopolymer-Based Nanocomposites](#)
- [Nanofibers and Nanomembranes of Biopolymers](#)

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Nanofibers and Nanomembranes of Biopolymers

21

Vinod V. T. Padil

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Abstract

Biopolymers contribute from naturally occurring as well as synthesized formats. This chapter exemplifies the significant nature of biopolymers, which originated from various sources such as plants, microbial, algae, animal, or botanical sources. These biopolymers are manufactured into electrospun fibers and membranes via multiple techniques. The processing parameters and ensuing fibers were characterized by many sophisticated analytical, microscopic, and spectroscopic methods. The numerous applications of membranes and fibers were also

V. V. T. Padil (✉)

Amrita School for Sustainable Development (AST) / Amrita Vishwa Vidyapeetham, Amrita University, Kollam, Kerala, India

e-mail: vinodvtp@am.amrita.edu

systematically demonstrated in various fields such as energy storage, food packaging, catalysis, biomedical, antimicrobial, bioelectronics, chemical sensors, water filtration, and others.

Keywords

Biopolymers · Electrospinning · Nanofibers: membranes · Water purification · Energy harvesting/storage · Biomedical

Introduction

Biopolymers are classes of materials originating from natural resources or biosynthesized materials obtained from living organisms or chemical derivatives of biological materials. These materials, which are nontoxic, biodegradable, and economically feasible, have a wide range of potential uses in the sectors of electronics, medical devices, energy, food packaging, and the environment. The biopolymers are classified according to their sources and presented in Fig. 1. Biopolymers are a diverse group of materials obtained from biological sources, including trees/plants, animals, and microbes, and also involve materials chemically synthesized from biological sources like sugars, oils, proteins, fats, exudates, and resins (Venkateshaiah et al. 2020).

Biopolymers have several unique characteristics, including renewability, natural abundance, low cost, unique structures, nontoxicity, biodegradability, noncarcinogenic,

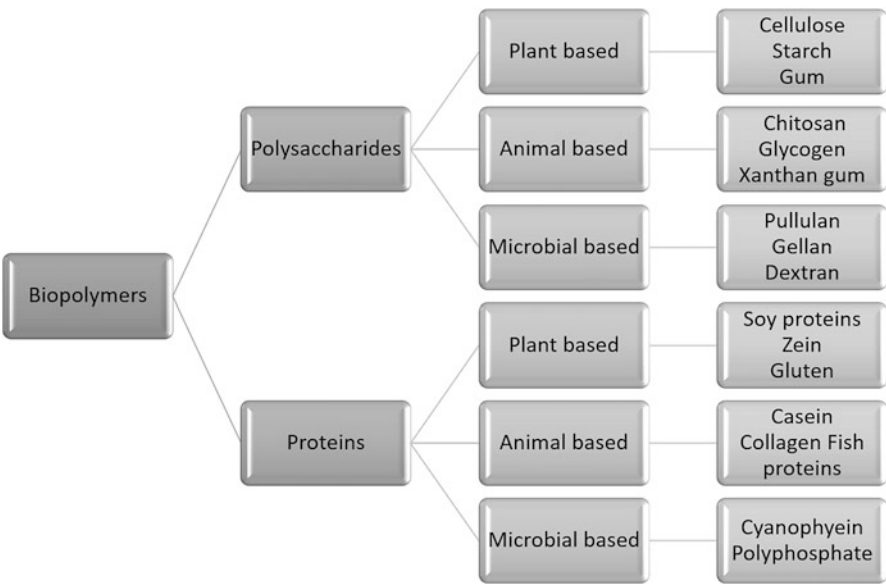


Fig. 1 Classification of biopolymers

nonthrombogenic, and biocompatibility, and are carbon neutral (Gheorghita et al. 2021). Nature-derived biopolymers, in contrast to conventional polymers with a simpler structure, have a broad range of structural complexity, which are frequently dependent on several factors such as the species, source, technique of extraction, and the age of the plant. However, the commercialization of these natural polymers is hindered by economic and engineering constraints. Nevertheless, due to their versatility, abundance, and low cost, biopolymers hold tremendous potential to replace petroleum-based conventional polymers in various applications, including packaging, biomedical, textile, structural materials, cosmetics, drug delivery, and food technology.

Biopolymers are classified using various criteria in the literature; however, they may be roughly classified into natural and synthetic biopolymers. Natural biopolymers are those that are acquired directly from nature while synthetic biopolymers are those that involve human intervention and chemical synthesis techniques to obtain the polymers. Natural biopolymers extracted from biomass can be further subclassified as polysaccharides, proteins, and lipids. Polysaccharides are the most abundant and diverse group of biopolymers available on the planet and are an essential component that performs various biological functions including communication between the cells, molecular recognition, and cell adhesion (Mohammed et al. 2021). These are complex carbohydrate polymers composed of monosaccharide units connected through glycosidic linkages forming linear or branched chain molecules of various lengths. They are either glycosidically connected sugar residues or covalently bound to other structures such as amino acids, peptides, and lipids. The physical and chemical characteristics of the polysaccharides are influenced by the monosaccharide unit type, chain configuration, molecular weight, branching, type of linkages, and its anomeric configuration (Mohammed et al. 2021). The most common polysaccharides obtained from biomass are cellulose, starch, chitin/chitosan, and gums.

The various fabrication techniques have been developed such as microextrusion, microfluidic spinning, and electrospinning (conventional, dry, and wet) to produce fibers and membranes. The ensued fibers showed various kinds of applications with respect to their fiber diameter and morphology.

Nanofibers: Preparation Techniques and Properties

During the last few decades, natural nanofibers (NFs) have shown outstanding performance in various applications, including energy, environment, water purification, sensor technology, tissue engineering scaffolds, wound dressings, drug delivery, healthcare, etc. (Wang et al. 2023; Jain et al. 2020). Electrospinning is one of the most effective and sophisticated methods for the large-scale production of nanofibers with a high surface area, consistent porosity, and high efficiency. The literature has extensively reported the electrospinning process parameters, such as system and process variable requirements. This method creates flexible, nano- to microscale fibers with a high surface-to-volume ratio, different sizes and geometries, and high membrane porosity (Jain et al. 2020). In this chapter, the multiple biopolymers outlined in Fig. 1 and their engineering variables that influence the morphology of

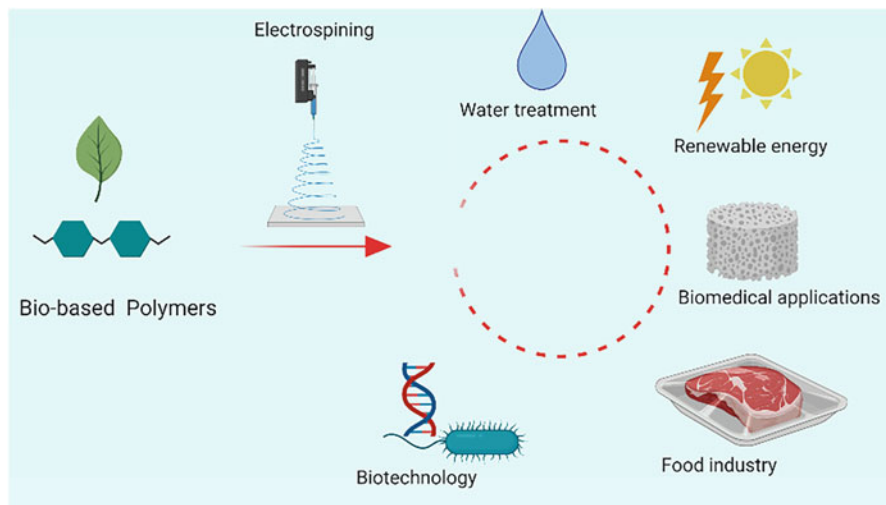


Fig. 2 Biopolymer-based electrospun fibers, membranes, and their applications. (Reproduced with permission from Ref. (Akshay Kumar et al. 2021))

nanofibers or membranes, systems, and processing parameters, and the effect of solvents' applications (outlined in Fig. 2) and other various types of electrospinning techniques have been discussed.

Nanofibers Based on Cellulose

Cellulose extracted from plants is profusely available on the earth. Cellulose, one of the abundant earth's materials, is a renewable product with exceptional qualities like worthier physical robustness, biocompatibility, and excellent environmental credentials. It has tremendous potential for industrial, pharmaceutical, environmental, and medical applications (Wei et al. 2014; Credou and Berthelot 2014). The important structural links in cellulose are represented by $-(1-4)\text{-D-glucopyranosyl}$ residues, and the molecule's β -sheet symmetry harbors intra- and intermolecular hydrogen bonds (Klemm et al. 2005). High-crystalline native cellulose is insoluble in aqueous and common organic solvents (Moon et al. 2011). Cellulose is derived mainly from plant materials, viz., wood pulp, disintegrating wood chips, cotton, cotton linter, and other fiber pulps courtesy of photosynthesis. In contrast, other sources, e.g., animals, algae, fungi, bacteria, invertebrates, and amoeba, also generate cellulose (Valencia et al. 2019; Eyley and Thielemans 2014; Prakash Menon et al. 2017). They can be used to make paper, casing, sanitary items, rayon, cellophane derivatives, textile materials, additives, medicinal fabrics, and various other things (Hon 1994; Lejeune and Deprez 2010; Barhoum et al. 2017).

Electrospinning is the most promising method to generate cellulose nanofibers (Fig. 3).

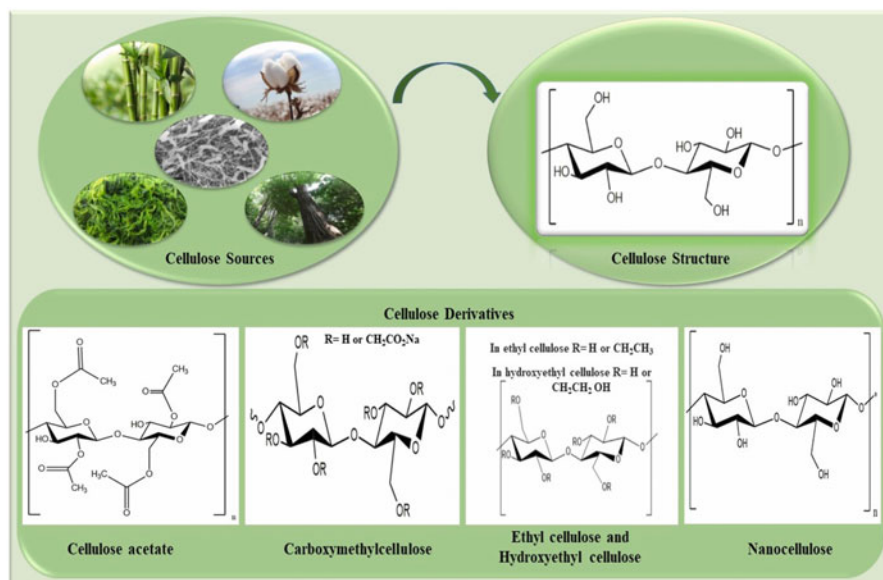


Fig. 3 The various sources of cellulose and their derivatives with formed structural attributes. (Reprinted from the permission from Ref. (Marques-Marinho and Vianna-Soares 2013; Mahsa Janmohammadi et al. 2023))

To create the corresponding nanofibers, many natural polymers rely on a “top-down” approach. Natural polymers are exposed to acid or alkali treatment to cleave the noncrystalline portions in their structures to achieve high solubility and homogeneity in diverse solvents. Furthermore, the smooth electrospinning of natural polysaccharides is skeptically influenced by variables including high solution viscosity, inter/intramolecular hydrogen bonding, gelling behavior, and peculiar rheological features (Moon et al. 2011; Wen et al. 2017).

Various physical, chemical, biological, and oxidative techniques are frequently employed to create cellulose nanofibers. The available approaches used to produce the appropriate cellulose nanofibers include ultracentrifugation, high-speed homogenization, ultrasonication, acid/alkali digestion, enzymatic degradation, and (2,2,6,6-tetramethyl piperidine-1-yl)oxyl radical (TEMPO) oxidation (Kousaku 2015). Cellulose extracted from bacteria and plant systems has also been successfully transformed into cellulose fibers (Prakash Menon et al. 2017; Eichhorn et al. 2010; Chirayil et al. 2014).

There are many methods to prepare the cellulose composite membranes, such as alkali treatment, polymerization, isocyanate grafting, acetylation, dewaxing, and periodate treatment (Zhang et al. 2022a) (Fig. 4).

The various methods used and their processing parameters and applications of cellulose-based nanofibers and membranes are deliberated in Table 1.

Fig. 4 Cellulose composite membrane preparation methods. (Reproduced with permission from Ref. (Zhang et al. 2022a))



Starch-Based Nanofibers and Membranes

Starch, generated as a storage material in most higher plant species, has been widely used in food and nonfood applications. It comprises complex polysaccharides – amylose and amylopectin – having α -D-glucopyranosyl (α -D-Glcp) units. The amylase-to-amylopectin ratios in starches vary depending on their source. Glycosyl residues [α -(1 \rightarrow 4) links in amylose and (1 \rightarrow 4)-linked α -D-Glcp units, as well as a high number of branch points α -(1 \rightarrow 6) connected in amylopectin], are found in wheat, corn, grain, potato, sugarcane, cassava flour, rye, barley, and oats (Dziedzic and Kearsley 1995; Xie et al. 2013; Li et al. 2014; Ambigaipalan et al. 2011; Stephen et al. 2006). Aside from dietary uses, starch and its chemical derivatives with carboxyl, acetyl, and hydroxypropyl functional groups produced via cross-linking, grafting, and esterification have received a great deal of attention in the environmental and biomedical applications (Moad 2011; Chen et al. 2015; Ashogbon and Akintayo 2014; Tharanathan 2005). Recently, a detailed review on the chemical derivatives of starch, particularly etherification and esterification, was published (Gilet et al. 2018; Meli et al. 2010).

The literature and patented technologies have documented the electrospinning of starch and its derivatives (Liu et al. 2017; Hemamalini and Giri Dev 2018; Woranuch et al. 2017; BeMiller and Whistler 2009). Polymers' diverse nonstarch materials, copolymers, (such as polycaprolactone (Jukola et al. 2008) polylactic acid (Sunthornvarabhas et al. 2011)), plasticizers, and cross-linkers blended with starch were successfully electrospun into fibers. The prerequisite for electrospinning is that the

Table 1 The various fabrication methods and their processing parameters and applications of cellulose-based nanofibers and membranes

Cellulose derivatives	Additive polymers/NPs/solvents or other materials	Fabrication process/properties	Application	Ref.
Cellulose acetate	Pullulan; <i>N,N</i> -dimethyl acetamide (DMAc) and dimethyl sulfoxide (DMSO)	Conventional/wet Electrospinning	Scaffold for tissue engineering	(Atila et al. 2016)
Cellulose membrane	Co ₃ O ₄ @Fe ₃ O ₄ nanoparticle	Blending	Perfluorocarbonic acid degradation	(Gao et al. 2022)
Cellulose membranes	Glutathione-capped gold nanoclusters	Casting method/electrostatic interaction	Biobased sensor-detecting Pb(II) and Hg(II) ions in water	(Jiang et al. 2022)
Cellulose carbamate membrane	Urea, <i>N,N</i> -dimethylacetamide (DMAc), sodium hydroxide, sodium hypochlorite (NaClO), and sulfuric acid (H ₂ SO ₄)	Solution casting	Antibacterial food packaging	(Zhang et al. 2022b)
Cellulose nanofiltration membrane	Chitosan, dimethyl sulfoxide (DMSO)–1-butyl-3-methylimidazolium acetate ([Bmim][OAc])	Phase inversion process	Oil/water separation	(Hardian et al. 2022)
Bamboo cellulose	Piperazine (PIP) and 1,3,5-trimesoyl chloride	Interfacial polymerization	Nanofiltration	(Li et al. 2018a)
Cellulose nanofibers	PAN/ PET/ <i>N,N</i> -dimethylformamide (DMF)	Infusion and electrospinning	Removing bacteria, viruses, and heavy metal ions	(Wang et al. 2013)
Ultrafiltration cellulose membranes	TEMPO-oxidized cellulose nanofibrils/poly (vinyl alcohol)	Casting and coating	Adsorption dyes, removal of BSA, and <i>E.coli</i> bacterium	(Aguilar-Sanchez et al. 2021)
Cellulose acetate nanofiber membranes	Acetone and DMAc	Electrospinning	Harvesting of electricity	(Zhang et al. 2022c)
Superhydrophobic cellulose membrane	Fe ₂ O ₃ , STA	Deposition and coating method	Water/oil separation and photocatalytic degradation of methylene blue	(Yin et al. 2021)

(continued)

Table 1 (continued)

Cellulose derivatives	Additive polymers/NPs/solvents or other materials	Fabrication process/properties	Application	Ref.
Cellulose acetate fiber-bead nanostructures	Lignin, dimethylformamide (DMF), and acetone and castor oil	Electrospinning	Lubricating uses	(Rubio-Valle et al. 2022)
Cellulose acetate	Polyvinylpyrrolidone, garlic extract, and acetic acid	Electrospinning	Bioactive molecules preservation	(Edikreshna et al. 2020)
Cellulose membranes	Cellulose nanocrystals	Additive printing	Separation of oil/water nanoemulsions	(Li et al. 2019)
Regenerated cellulose membranes	[TMGH]2+[OOCOCCH2CH2OCCO]2-/DMSO, ethanol, methanol, NaOH, and H2SO4	Casting and coagulation	Packaging, food, and agricultural industries applications	(Guo et al. 2021)
Cellulose membranes	Protein-derived Pyura chilensis	Top-down approach	Biocompatible for potential applications	(Quero et al. 2019)

polymer concentration be double that of the entanglement concentration for efficient electrospinning (McKee et al. 2004).

The method of developing electrospinning solutions by dissolving high amylose content starch (15% w/w) in a 95% aqueous DMSO solution (Kong and Ziegler 2014a) outperformed electrospinning starch with inclusion complexes (for example, palmitic acid) in ethanol or ethanol/water systems (Kong and Ziegler 2014b). Using DMSO as the solvent, Kong et al. (Kong and Ziegler 2012) assessed molecular entanglement, conformation, and shear viscosity, all of which are crucial factors in starch electrospinning. The results suggested that the starch concentration needs to be higher than 1.2–2.7 times the entanglement concentration.

Starch-based nanofibers were used in various applications. MWCNT/starch/PVA nanofiber webs were fabricated and carbonized onto CNT/C nanofibers and subsequently developed as electrochemical capacitor electrodes with high specific capacitance (170 F g^{-1}) and electrical conductivity (2.1 S cm^{-1}). Lancuški et al. produced electrospun fibers with sizes ranging from 80 to 300 nm by electrospinning high-amylose starch formate in aqueous formic acid. Furthermore, Xu et al. reported electrospun starch acetate fibers by dissolving it in formic acid.

In another study, high-amylose Hylon VII maize starch and starch-derived nanographene oxide (nGO) (via carbonization) were electrospun using formic acid as solvent (Wu et al. 2017), where the incorporation of nGO into starch improved fiber diameter, hydrophilicity, thermal stability, and electrospinnability. Li et al. (2018b) have demonstrated that starch-based fiber can spin centrifugally, and Jeong et al. (2016) reported on the utilization of carbon nanofibers (CNFs) generated from starch and carbon nanotubes (CNTs) in rechargeable batteries. The constructed electrode performed admirably (510 mAh g^{-1} after 30 cycles), which can be attributed to its huge surface area.

Pectin Nanofibers

Pectin is a naturally occurring heteropolysaccharide derived from terrestrial plant tissues that is primarily constituted of poly (α -1, 4-D-galacturonic acid) inserted with α -1, 2-L-rhamnopyranose unit (Maxwell et al. 2012; Yapo 2011; Williams et al. 2011). Citrus peel, apple pomace, and sugar beet pulp are the most common commercial sources of pectin. It is used in the food industry as a thickening, gelling, and stabilizing agent and has incredible potential in biomedical applications. Adding metal salts can increase the viscosity of pectin (Löfgren et al. 2006; Pina et al. 2015; Munarin et al. 2011, 2012). Pectin with PEO (polyethylene oxide) as a copolymer and Triton X-100 as additives were successfully electrospun into fibers in a DMSO/aqueous mixture (Cui et al. 2016). The aqueous solutions of pectin (5–8 wt.%) and PEO (5 wt.%) were mixed in various weight ratios (pectin/PEO: 60/40, 70/30, 80/20, and 90/10) before being blended with 1% and 5% Triton X-100 and DMSO, respectively. After that, the dispersions were mixed for 3 h before electrospinning. In the blends of pectin/PEO, all other combinations except the 90/10 ratio gave uniform and smooth nanofibers. The electrospinning of charged natural

polysaccharides (e.g., chitosan and alginate) proved difficult on their own, emphasizing the importance of copolymers, additives, and appropriate solvents. This is because such systems contain complicated molecular chain entanglements in solution and different rheological characteristics (Nie et al. 2009; Klossner et al. 2008; Stijnman et al. 2011).

Additionally, alginate/PVA electrospun fibers in combination with therapeutic (chemical) cargo (viz. Lidocaine, Neomycin, and Papain) have demonstrated their potential both as dressing materials and as media or conduits for transdermal delivery of an antibiotic drug (ciprofloxacin) (Pegg et al. 2014; Kataria et al. 2014). In another study, nanofibers of alginate/PEO and lavender essential oil were used to cure UV-induced skin burns. They showed good antibacterial activity against *S. aureus* exhibiting anti-inflammatory properties under both, in vivo and in vitro experimental conditions (Hajiali et al. 2016). Composite electrospun fibers based on alginate (2 wt. %)/PVA (16 wt. %) in a 1:1 blended solution of PVA/alginate with ZnO NPs (0.5, 1.2, and 5%) were fabricated by Shalumon et al. (2011). Higher thermal stability, antibacterial, and cytotoxicity attributes were exhibited by alginate/PVA/ZnO composite fibers compared to the pristine sample (PVA/alginate fibers).

Fabrication of “Green” Electrospun Fibers Based on Tree Gums

The development of “green electrospinning” technology requires nontoxic and inexpensive biodegradable ingredients and environmentally friendly solvents. Green technology aims to environmental protection in contrast to the current electrospinning method, which requires destructive and hostile organic solvents.

Electrospun fibers and membranes based on natural renewable resources are continuously being developed for energy and environmental purposes. To create electrospun fibers and membranes, “green” electrospinning technology focuses on developing nontoxic, low-cost, and environmentally friendly polymers and solvents (such as water or ionic solvents). Natural biopolymers such as chitin, chitosan, cellulose, starch, gums, etc., have been successfully electrospun into fibers. Many of them are blended with other natural or synthetic fibers to formulate their blend to make uniform and smooth fibers.

Tree gums exemplify sustainable and renewable biomacromolecules abundantly available from nature and possess excellent biodegradability, nontoxicity, low cost, physicochemical properties, structural attributes, and diverse functional groups. Gum hydrocolloids, often known as tree gum polysaccharides, are obtained as the natural exudates of different tree species and represent a largely unexplored source of valuable natural products. Exudate gums have wide range of applications in food and pharmaceutical industries as stabilizers, thickening agents, emulsifying agents, and binding agents. Recently there is a growing interest in the use of these exudate gums for nonfood applications including biomedical, food and pharmaceutical packaging, and energy and environmental applications. The discovery of novel uses for these gums has piqued people’s attention in recent years.

Abundant availability and biodegradability coupled with nontoxicity and low cost can make these materials ideal for the fabrication of packaging films to replace conventional plastics. However, these exudate gums have intrinsic limitations including high hydrophilicity, poor film-forming ability, and low mechanical properties. To overcome these drawbacks, researchers employed different strategies including chemical modifications, blending with different nanomaterials (graphene oxide, nanocellulose), 2D nanoclay coating, and blending with other natural polymers. The obtained blended component demonstrated significant improvement in the physicochemical, mechanical, thermal, and barrier properties while retaining the biodegradability of the gums.

Additionally, many nanotechnological and biotechnological approaches are recently used as novel and green applications to valorize tree gum wastes and other bio-wastes. The carbon-rich structure of the gum wastes has been utilized in the growth of carbon nanostructures with exceptionally high surface area. These carbon nanostructures have been further used in the development of energy harvesters. The large functional groups of the gums have been used in the product of graphene-supported nanoparticles for environmental remediation applications.

Electrospinning of Tree Gum Exudates

Many researchers have reported on the electrospinning of tree gums and their blended solutions containing various natural or synthetic polymers in the last 5 years. The system and process parameters for the electrospinning of tree-gum-based nanofibers are the critical criteria to strictly follow to produce nanofibers of smooth beads and uniform diameters (Table 2).

Electrospinning of Pullulan (PU)

PU is a commercial, neutral carbohydrate polysaccharide that is widely used in a variety of sectors, including the production of cosmetics, drugs, and food. It is a nonionic microbial polysaccharide that the fungus *Aureobasidium pullulans* produces. Pullulan's structure is α -(16)-linked (14)- α -D-triglucoside (maltotriose), and it is dependent on water solubility and structural flexibility.

Deniz Atila et al. (2015) electrospun PU and cellulose acetate in a variety of ratios (80/20, 50/50, and 20/80 wt/wt) to create 3-D fibers. DMAC/DMSO (55/45 v/v%) was the solvent used for electrospinning, and the effects of different parameters have been researched. The fiber diameter, porosity, and thickness of the fiber scaffolds of the resulting nanofibers were all affected by the amount of PU in the blended mixture. The 50/50 wt/wt ratio was discovered to be the ideal ratio, demonstrating the efficacy of 3-D scaffolds in tissue engineering.

Pullulan, PVA, and rutin mixes have been electrospun into mats for use in ultraviolet (UV) resistance applications, and the fiber homogeneity and diameter have been measured. Rutin's presence and concentration also blocked UV radiation,

Table 2 Electrospun fibers based on tree exudates gum composites fibers and membranes and their properties

Tree exudates	Electrospinning techniques/ parameters	Fiber properties and applications	Reference
Gum Arabic	Solution blending with PVA and Au nanostructures	Fiber diameter = 148- to 303 nm Biomedicine for cancer treatment	(Serio et al. 2021)
Gum Arabic	Blending with PVA and <i>L. acidophilus</i>	189.9 nm (fiber diameter) Delivery of probiotics for the development of functional foods	(Fareed et al. 2022)
Gum Arabic	Blending with graphene oxide and PVA	100 nm Dye removal from water	(Silvestri et al. 2019)
Gum Tragacanth (GT)	Blend electrospinning of gum Tragacanth/polyvinyl alcohol/ molybdenum disulfide composites with tetracycline	>100 nm (nanofiber diameter) Antibacterial wound healing	(Khaledian et al. 2021)
GT	Chitosan and gum tragacanth via cryotropic gelation	96.56–30.21 μm Scaffold for tissue engineering	
Gum Karaya	Blend electrospinning with PVA	100–300 nm (fiber diameter) Adsorptive removal of nanoparticles from water	(Padil and Černík 2015)
Gum Kondagogu	Blending and electrospinning PVA/PEO/Water	200–400 nm Various applications	(Bilal et al. 2021)

lowering UVA and UVB radiation transmission by 5% (Qian et al. [2016](#)). By using PU and whey protein conjugates, the techniques of electrospinning and electro-spraying have been successfully used to create nano-/micro capsules for the probiotic encapsulation of bifidobacterium (Senthil Muthu Kumar et al. [2019](#)).

Dextran Nanofibers via Electrospinning

A polysaccharide obtained from bacteria is dextran. It has a linear structure made up of repeated α -1,6-linked D-glucopyranosyl units containing α (1 \rightarrow 2)-, α (1 \rightarrow 3)- or α (1 \rightarrow 4)- glycosidic linkages connecting the backbone to branches of shorter chains of D-glucose (Cheetham et al. [1990](#); Naessens et al. [2005](#); Gulke [1989](#)).

Due to dextran's great water solubility, its solutions exhibit Newtonian fluid behavior. The concentration, temperature, and molecular weight of the solution significantly affect its viscosity. Dextran has been utilized as a food additive in the pharmaceutical and cosmetic industries due to its biodegradability and biocompatibility. Additional uses in biomedicine include drug therapy, protein and enzyme

imaging agents, and plasma volume expanders (Hennink and van Nostrum 2012; Lipman 1993). Dextran's main functional group, like -OH, is in charge of conjugating other molecules. Most notably, dextran is easily blended with hydrophobic polymers (like PLGA) to create composite electrospun fibers since it is soluble in both water and organic solvents. Dextran's mechanical strength, the swelling characteristics of water, and biological activity can all be managed in this way.

Wang et al. conducted electrospinning of dextran to create random polymer fibers with different molecular weights (50, 100, 500, and 2000 kDa) and polymer concentration, influencing fiber diameters and structures. The variation of morphology is dependent on attention and applied voltages. Using fiber orientation to control cell adherence and spreading by electrospinning has been made possible by this research (Wang et al. 2018). By electrospinning acetylated dextran, Borteh et al. created a scaffold for the release of resiquimod (4-amino-, -dimethyl-2-ethoxymethyl-1Himidazo[4,5-c]quinoline-1-ethanol). The synthesized acetylated dextran scaffold is a strong contender for biomedical applications due to its cytocompatibility, flexibility, tenability, and controlled drug-releasing ability (Borteh et al. 2013). The process parameters and solvent systems used to electrospin dextran fibers in water, DMSO/water, and DMSO/DMF have been optimized by Jiang et al. (2004). The bovine serum albumin (BSA) or lysozyme 10% (w/w) addition had no effect on the electrospun dextran fibers. Additionally, the viability of creating electrospun membranes by utilizing methacrylate dextran to photo-crosslink in the solid state has been examined.

Electrospinning of Hyaluronic Acid

An anionic, nonsulfated glycosaminoglycan, hyaluronic acid (HA) is composed of chains of repeating units that connect [β -1 \rightarrow 3] D-glucuronic acid and [β -1 \rightarrow 4] N-acetyl-D-glucosamine. All higher animal species have it in their extracellular matrix of tissues, and certain microorganisms also make it (Laurent and Fraser 1992; DeAngelis 1999; Campoccia et al. 1998; Bulpitt and Aeschlimann 1999; Shu et al. 2004). Potential uses for HA and its derivatives include tissue engineering and drug delivery in medicine, as well as biotechnology, pharmaceuticals, and cosmetics (Purcell et al. 2014; Makvandi et al. 2019, 2020).

The sulfated HA macromers were electrospun into fibers by Purcell et al. (2014). These nanofibers could be used for tissue engineering and drug delivery, specifically. They could also be used to convey heparin and cause protein interaction.

Li et al. (2006a) investigated the formation of HA and HA/gelatin electrospun fibers (with diameters of 200 nm and 190–500 nm, respectively) and the electrospinning properties of HA and blended mixes of HA/gelatin in N, N-dimethylformamide (DMF)/water-mixed solvent systems. Another study that involved electrospinning gelatin/HA blends into electrospun fibers demonstrated that the addition of HA could enhance the electrospinning and processability of aqueous gelatin solutions, increasing the viscosity and conductivity of the blended solutions (Li et al. 2006b).

Heparin Electrospun Fibers

A naturally occurring glycosaminoglycan, heparin, is composed of D-glucuronic acid, L-iduronic acid, and D-glucosamine units and has a high concentration of sulfonate and carboxylic functions. Heparin has a number of important biomedical uses that depend on its antiviral, anticoagulant, and anticancer properties. Additionally, it encourages angiogenesis, the growth of new blood vessels, which is essential for the development of functioning tissues (Capila and Linhardt 2002; Mizrahy and Peer 2012; Liu et al. 2014; Cheng et al. 2014). Vascular tissue engineering and drug delivery systems are two important areas where heparin-conjugated electrospun nanofibrous scaffolds are used. Feng et al. (Feng et al. 2017) investigated the creation of core-shell-type fibers using electrospinning of heparin, Rosuvastatin calcium (a drug used to treat hyperlipidemia), and poly(L-lactide-co-caprolactone) blended solutions with the appropriate solvents. The diameter distribution, water contact angles, and cell survival of the nanofibers are also summarized. The study demonstrated that electrospun fibers can be produced, retain bioactivity, and be used as a framework for stent grafts to treat aneurysms while also releasing heparin and rosuvastatin calcium in a sustained manner.

Several heparin-based electrospun scaffolds have been constructed. Casper et al. (2005) monitored the electrospinning of a combination of PEO and heparin or PEG-low molecular weight heparin in aqueous media or DMF. In another study by Kwon et al. (Kwon and Matsuda 2005), nanofibrous heparin-TBA (tri-n-butylamine) were conjugated via electrospinning poly (L-lactide-co- ϵ -caprolactone) or PLCL with the tri-n-butylamine salt of heparin, using various blended ratios. Luong-Van et al. (2006) incorporated heparin into electrospun poly (epsilon-caprolactone) or PCL fiber mats in the solvent systems. It was found that the fibers could release heparin over 14 days without triggering an inflammatory response.

In another study, heparin was conjugated via hydrogen bonding with SF (silk fibers), and the electrospun SF-heparin nanofibrous scaffolds were suitable for biomedical applications. The electrospun fibers of PCL and heparin-conjugated scaffolds loaded with FGF2 (Fibroblast Growth Factor 2) or VEGF (vascular endothelial growth factor) have been successfully utilized for vascular tissue engineering (Ye et al. 2011, 2012). Wang et al. (2011) demonstrated that electrospun heparin/gelatin fibers could be prepared using a gelatin/acetic acid solution, which was then cross-linked with a glutaraldehyde/ethanol solution. Coaxial electrospinning was used to create a core-shell fibrous membrane, with PCL serving as the core section and cationized gelatin (CG) as the shell material. Onto these core-shell fibrous scaffolds, macromolecular bioactive substances, such as FITC-BSA and FITC-heparin, may be adsorbed. A core-shell fibrous membrane with cationized gelatin (CG) as the shell material and PCL as the core section was established via coaxial electrospinning (Lu et al. 2009). Onto these core-shell fibrous scaffolds, macromolecular bioactive substances, such as FITC-BSA and FITC-heparin, may be adsorbed.

Chitin and Chitosan Nanofibers and Membranes

The animal polysaccharide chitin is the second most prevalent biopolymer after cellulose, found in nature, and has a wide range of uses in the domains of biocatalysis, energy storage, filtration, biosensing, and biomedicine (Jayakumar et al. 2010; Ding et al. 2014; Schiffman and Schauer 2008). When compared to its derivative (chitosan), chitin is made up of (1, 4)-linked *N*-acetyl- β -D-glucosamine, while chitosan is made up of (1–4)-linked 2-acetamide-2-deoxy- β -D-glucopyranose and 2-amino-2-deoxy- β -D-glucopyranose.

One major setback of the electrospinning of chitin and chitosan is the inability to precisely select the optimal solvent for processing. Additionally, the polycationic structure of chitin and chitosan as well as the existence of hydrogen bonds could prevent them from electrospinning smoothly (Ding et al. 2014). Chitin has been solubilized using a variety of systems that contain acidic components, such as methane sulfonic acid, hexafluoroisopropanol, and lithium chloride/dimethyl acetamide (Muzzarelli 2011). Ionic liquids have lately been investigated as promising solvents for chitin and chitosan. The prime goal was to ensure the two aforementioned animal-sourced polysaccharides' physicochemical properties were not to be altered prior to their usage in electrospinning (Wang et al. 2012; Zakrzewska et al. 2010). Chitin and chitosan, as well as their nanofibers, have been widely used in biotechnological fields due to their essential biological characteristics (Ignatova et al. 2009; Zhong et al. 2010).

Three different varieties of chitin – P-Chitin (Temperature-Preprocessed Shrimp Shells), R-Chitin (Unrefined Shrimp Shells), and PG-Chitin (Useful Grade Chitin) – were electrospun using an ionic liquid (1-ethyl-3-methylimidazolium acetate) by Zavgorodnya et al. (2017). Chitin content and viscosity were found to be the main determinants of the electrospinning process in studies of a multitude of factors, including conductivity, polymer concentration, viscosity, and surface tension of the electrospinning fluid. Contrary to what has been reported elsewhere (Jarusuwannapoom et al. 2005; Pattamaprom et al. 2006), solution conductivity and surface tension are not the dominant factors when volatile organic chemicals are utilized.

For application in oil-in-water emulsion separation, Wu et al. created a filtering membrane using chitin nanowhiskers and poly *N*-isopropyl acrylamide-co-*N*-methylol acrylamide (PNIPAm-co-NMA). By electrospinning PNIPAm-co-NMA and ChNW at a concentration of 15 wt% in a water/THF mixture, the electrospun membrane (PNIPAm-co-NMA)/ChNW was generated (in a 1:2 solvent ratio). It outperforms alternative methods by five-fold in terms of cleaning oil-in-water emulsions under high salinity and wide pH range conditions (Croisier et al. 2015).

Park et al. prepared a blend of chitin/poly (glycolic acid) nanofibers using HFIP as a solvent via electrospinning (Park et al. 2006a). The scaffolds, which might be used in tissue engineering, demonstrated outstanding cell adhesion and spreading capabilities for regular human fibroblasts. Chitin-/silk-blended nanofibers were also produced using the same solvent (Park et al. 2006b), where the resulting blended nanofibers exhibited 140 nm average diameter. The diameters of the nanofibers

dropped from 920 to 340 nm as the chitin concentration of the composite compositions increased.

Chitosan nanofibers coupled with the polyelectrolyte poly(acrylic acid) (PAA) were produced by electrospinning a ternary solvent solution of formic acid, glacial acetic acid, and water (Zhang et al. 2018). The mechanical and swelling features of the films and fibers are pH dependent, and hence the transport and release of pharmaceuticals is the enticing potential of these materials. It has also been observed in another study that the degree of deacetylation (DD) affects the gelation of chitosan (in relation to particle size and the conductive, dielectric, and rheological characteristics under electric field).

Using the electrospinning process, Koosha et al. (2015) created Na-montmorillonite (MMT) nanoclays and chitosan/poly (vinyl alcohol)-based nanofibrous nanocomposites. The viability of the A-431 cell line, which was discovered to be properly adherent to the surface of the mats, was tested using an MTT assay to determine the cytotoxicity of nanocomposite mats. The results revealed that the mats had no adverse impacts on the viability of the cell line. Croisier et al. reported a method for electrospinning a mixture of PCL with a poly (MMA-MA) copolymer of methyl methacrylate and maleic anhydride, resulting in negatively charged electrospun fibers with chitosan coating (Croisier et al. 2015). The carboxylic acid was deprotonated in neutral or basic aqueous conditions to achieve this.

Electrospun chitosan/polyethylene oxide (PEO) fibers containing silver nanoparticles (Ag NPs) were created by Annur et al. (2015) by reducing AgNO_3 on the surface of the nanofibers using an argon plasma treatment. The data showed an improvement in the antibacterial efficacy of the nanofibers after argon plasma treatment, and the average diameter of the Ag NPs deposited on the surface of the nanofibers was 1.5 nm. This is explained by the strong contact between the amino group of chitosan and the silver nanoparticles, which resulted in the silver nanoparticles remaining exposed on the nanofiber surfaces for an extended period of time.

Chitosan-based nanofibers are employed in water purification purposes as well, e.g., removal of heavy metal ions. By electrospinning chitosan, PVA, and Fe (III) in aqueous/acetic acid, Chauhan et al. produced biopolymeric nanofibers, which were further used to remove As (III), As (V), Cr (VI), and F⁻ ions successfully from contaminated water (Chauhan et al. 2014). Additionally, they showed promising antibacterial potential against *Escherichia coli*, a Gram-negative, optional anaerobic, rod-shaped coliform bacterium. Chen et al. (Chen and Huang 2016) exploited N-maleoyl-functionalized chitosan (MCS)/PVA in an aqueous medium to electrospin ecofriendly fibers. Through using allyl disulfide as the cross-linker and UV radiation to cross-link the fibers, hazardous chemicals were avoided. Suitable antibacterial agents were added to the produced nanofibers, which were then used in applications for wound healing. Makaremi et al. (2016) created electrospun PAN nanofibrous membranes with a layer of

electrospun chitosan and functionalized zinc oxide (ZnO) nanoparticles (Cs). Improved mechanical, antibacterial, and water-filtering performance was the objective. The conjugated fiber layers showed high mechanical characteristics (tensile strength and Young's modulus) after the addition of chitosan fibers. The adsorption capability of Cr(III) was further improved by the addition of ZnO NPs. Furthermore, the high water permeability and antibacterial effectiveness of this sandwiched membrane suggested that it was suitable for water filtration. Desai et al. (2008) produced uniform-sized electrospun nanofibers of chitosan with a small amount of PEO (5%) using acetic acid as the solvent high-durability chitosan mats (up to 6 months) were prepared by Eroglu et al. (2012) which support algal growth and removal of nitrate ions from water. Horzum et al. (2010) accomplished the electrospinning of chitosan in 1,1,1,3,3,3-hexafluoroisopropanol (HFIP), the ensuing electrospun fibers being utilized for sorption of Fe(III), Cu(II), Ag(I), and Cd (II) ions from aqueous solutions. Lu et al. (2012) prepared graphene impregnated with chitosan nanofibers with antibacterial efficiency for application in wound dressing.

Coaxial electrospinning was utilized to regulate L-ascorbic acid/poly(vinyl alcohol)-chitosan core-shell nanofibers; acetic acid was employed as the chitosan shell and ethanol/propylene glycol/water as the L-ascorbic acid core solution. It assessed how well the model medicine ascorbic acid was released from the core-shell fibers. The outcomes showed that functionalizing such materials for transdermal medication delivery devices is appropriate (Najafi-Taher et al. 2015). Pakravan et al. (2012) produced core-shell-structured PEO-chitosan nanofibers by coaxial electrospinning with the inner layer and outer sphere membrane consisting of PEO. The chitosan was fabricated in acetic acid (solvent), and PEO was later washed out using water to sustain the core-shell structure of the chitosan nanofibers.

Electrospinning of Gelatin

Gelatin, derived from animal tissue, possesses a high level of denatured collagens. Mammalian source, e.g., pig skin and bovine hides, is the primary source of gelatin production. Gelatin has many applications in the pharmaceutical industry, for instance, the main ingredient of hard capsules that melt in water (at $> 30^{\circ}\text{C}$) and release their therapeutic agents (Sadat-Shojai et al. 2016).

Hydrogels have been exploited as possible scaffolds for demanding tissue-engineering applications. However, their low mechanical properties and bioactivity make them unsuitable for hard tissue. One strategy is to improve their physical properties by adding electrospun fibers. Having this in mind, Mehdi-Sahad and coworkers (Sadat-Shojai et al. 2016) developed a three-layered hybrid scaffold based on hydroxyapatite fibers between two layers of methacrylate gelatin/hydroxyapatite (Table 3).

Table 3 Various proteins electrospun fibers using solvents or additives

Proteins	Solvents or additives	Ref.
Gelatin	PCL/TFE, acetic acid, TFE, PHBV/HFIP, water, PLGA/TFE, and nylon-6/formic acid; acetic acid, HFIP; ethyl acetate	(Xue et al. 2014; Gomes et al. 2015; Zhang et al. 2006; Wang et al. 2016; Tonda-Turo et al. 2013; Song et al. 2008)
Collagen	Water/ethanol/ salt, PHBV/HFIP, and AA/DMSO	(Wang et al. 2016; Punnoose et al. 2015; Rnjak-Kovacina et al. 2012)
Elastin	HFIP, collagen/HFIP	(Rnjak-Kovacina et al. 2012; Araujo et al. 2014)
Soy	PVA/water; PEO/water and PEO/HFIP	(Cho et al. 2010; Ramji and Shah 2014)
Zein	Ethanol, isopropanol	(Erdogan et al. 2015)
Whey	PEO/water	(Colín-Orozco et al. 2015)
Amaranth	Pullulan/formic acid	(Aceituno-Medina et al. 2015)

Electrospinning of Collagen

About one-fourth of the total protein in the human body belongs to this class of fibrous proteins (Gelse et al. 2003). These proteins maintain internal organs, connect tendons and muscles, and are crucial for the structure of cartilage, skin, bones, and teeth. The triple-helical structure of collagens is remarkably similar across a wide range of animal species, and they are relatively nonimmunogenic. These factors drive research into electrospun collagens for use in tissue engineering, wound healing, and other broad biomedical applications.

However, electrospinning collagens have shown to be challenging. It was necessary to find a solvent that could dissolve the protein at concentrations suited for bead-free fiber creation, permit quick drying of fiber mats, and be compatible with copolymers for improving mechanical qualities. According to Zeugolisa et al., the fluoroalcohol-induced dissolution of collagen causes significant protein denaturation in fibers (Zeugolis et al. 2008). Wnek and colleagues revealed that basic mixes of phosphate buffered saline and ethanol can facilitate electrospinning and protein dissolution while maintaining triple-helical structure (Dong et al. 2009).

Conclusion

The successful electrospinning of abundant biopolymers from plant, animal, bacterial, and seaweed sources has been implemented worldwide. The methodology produced electrospun fibers of uniform dimensions, making use of mainly natural materials, a proper choice of solvents (aqueous/organic), and the suitable system and processing parameters. Inadequate evaluation tools for measuring stability, fiber diameter, aspect ratio, and morphology have been a significant drawback of this technology. Another difficulty was that it was difficult to determine which particular

application would suit the resulting nanofibers best because of their varied physical, chemical, and mechanical properties.

A variety of natural biomaterials and their electrospun fibers have been widely used in energy, environmental, and medicinal applications (such as scaffolds for tissue engineering, drug delivery, and reduce inflammation) by virtue of their functional groups with medicinal value available in their structures, biocompatibility, nontoxicity, and biodegradable nature. The commercialization of these goods in packaging materials, bioengineering, and disposable sanitary items is essential for the enormous possibilities for natural polymer-based electrospun fibers. In this context, the newer nanofiber-derived goods will be promoted by academic and business institutions, as well as through their improved network and interaction. The use of biopolymers-based membranes and nanofibers will allay safety worries, which will make them highly sought after in a variety of industries, especially the medical, food, and pharmaceuticals.

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Part VI

Applications of Biopolymers

Biopolymer Based Soil Treatment for Geotechnical Engineering Applications

22

Evangelin R. Sujatha  and Brendan C. O’Kelly 

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Abstract

Biopolymers (natural polymers) are exuded by micro- and macro-organisms (bacteria, plants, etc.) and have been shown to affect the geotechnical engineering behavior and properties of different soil types for low dosage levels. The long-chain structure of biopolymers and certain constituting chemicals (e.g., hydroxyl, ester, or amines) supply adhesive forces that help coating and binding together the soil particles. This chapter presents a review of the state of the art in biopolymer-based soil treatment (BBST), aimed at focusing more attention on the various potential uses of biopolymers, as alternatives to conventional (cement and lime) binders, for a variety of applications in the geotechnical and geoenvironmental engineering sectors. Compared to conventional binders, the benefits of BBST

E. R. Sujatha

Centre for Advanced Research in Environment, School of Civil Engineering, SASTRA Deemed to be University, Thanjavur, India

e-mail: sujatha@civil.sastra.edu

B. C. O’Kelly (✉)

Department of Civil, Structural and Environmental Engineering, Trinity College Dublin, Dublin, Ireland

e-mail: bokelly@tcd.ie

include its low environmental impact, non-toxicity, and non-secondary pollution. This chapter also discusses various challenges and issues related to the field application of BBST and identifies various research needs.

Keywords

Biopolymer-treated soil · Geotechnical properties · Soil improvement

Abbreviations

BBST	Biopolymer-based soil treatment
CG	Carrageenan gum
CH	Chitosan
CMC	Carboxymethyl cellulose
GG	Guar gum
LL	Liquid limit
PAM	Polyacrylamide
SA	Sodium alginate
SSA	Specific surface area
UCS	Unconfined compressive strength
XG	Xanthan gum

Introduction

The geotechnical engineering sector consumes vast amounts of chemical additives (conventionally cement and lime), as binders, for various ground improvement and soil stabilization applications. These chemical additives permanently alter the treated soil environment, and they can also result in pollution of the groundwater. Biological methods represent green and sustainable alternatives, being broadly categorized as: (i) mineral precipitation – that is, methods that produce mineral precipitation in the pore voids to increase the soil particle bonding and/or reduced the soil permeability; (ii) mineral transformation – that is, methods that transform clay minerals from one form to another; and (iii) biopolymer-based soil treatment (BBST) that can improve the strength, and reduce the permeability, of the treated soil through ionic bond and hydrogel formation. The focus of this chapter is on the BBST.

Biopolymers are non-toxic and non-polluting polymers, produced naturally by microorganisms, animals, and plants, or synthesized chemically from materials of biologic origin, including sugars, amino acids, oils, etc. (Kavazanjian et al. 2009). Based on their composition, biopolymers can be classified as polyesters, proteins, or polysaccharides (Nair and Laurencin 2007); the latter being an important fraction of soil organic matter. As such, polysaccharide additives may be considered more suitable for BBST field applications, particularly those designed to improve the erosion resistance of surficial soil layers (Rabiei et al. 2022). Polysaccharides are produced from bacteria, fungi, plant and algae, and animal sources. Those produced from bacteria include xanthan gum (XG), gellan gum, dextran, levan, and curdian.

Common fungal polysaccharides include yeast glucans, pullulan, and elsinan. Examples of polysaccharides from plant and algal sources are starch, cellulose, agar, guar, and alginate, while those from animal sources include chitosan (CH), chitin, polyphenols, and hyaluronic acid.

Biopolymers are used extensively in various industries (e.g., food, textile, oil exploration, pharmaceuticals, and drug delivery) as thickeners, adhesives, lubricants, and adsorbents. The focus of this chapter is on BBST for various geotechnical and geoenvironmental engineering applications, including stabilization of weak soils, slopes, and excavations, for modifying marginal soil as contaminant barrier material and/or for compacted covers, and in resisting water- and wind-induced erosion of surficial soil layers. Table 1 lists the common biopolymer types presently employed in BBST to improve the geotechnical/geoenvironmental engineering behavior and properties of different soil types. Introducing biopolymers into the soil may be achieved by various methods, such as grouting, injection, mixing, and surface-spray application. The interaction between the soil particles and biopolymer additive is influenced by several factors, including the surface characteristics of the soil particles, the structural flexibility of the biopolymer (i.e., regarding van der Waal's interaction (Chang et al. 2015)), the presence of hydroxyl groups on the biopolymer and soil particles (i.e., regarding hydrogen bonding), and the presence of carboxyl groups (i.e., regarding ionic bonding between charged particles (Martin 1971)).

In addition to their ecofriendly nature, another major advantage of using biopolymers, as binders, for ground engineering applications is the relatively low dosage (as compared to cement and lime additives) required to achieve a comparable compressive strength of the treated soil. Furthermore, while most conventional grouts or other bonding agents are strong but brittle, and thus susceptible to fracture in a porous medium, BBST generally offers a more resilient response to stress concentration (Assadi-Langroudi et al. 2022). Biopolymers are stable over a broad range of temperatures and pH, with many of them forming viscous suspensions with water (Kumar and Sujatha 2021). Their properties, including a tendency to gel, pseudo-plastic behavior, and resistance to shear degradation, help in plugging the soil pore-void spaces (Wiszniewski and Cabalar 2014). However, it should be noted, as discussed later in the chapter, that biodegradability may limit biopolymer durability (Assadi-Langroudi et al. 2022). Grouting of biopolymers for ground engineering applications also requires higher inject pressures that depend on the biopolymer concentration/grout viscosity (Khatami and O'Kelly 2022).

BBST Applications in Geotechnical and Geoenvironmental Engineering

BBST has shown much promise in various geotechnical and geoenvironmental engineering applications. For instance, biopolymers XG and guar gum (GG) have been used to stabilize mine tailings (Chen et al. 2013) and for fugitive dust control. XG, GG, β -glucan, and carboxymethyl cellulose (CMC) have been successfully employed to improve the soil erosion resistance (Chang et al. 2019; Nikolovska et al.

Table 1 Properties of select biopolymers and their various applications in geotechnical and geoenvironmental engineering

Biopolymer	Source	Property	Application	References
Sodium-CMC	Ether derivative of natural cellulose from plants	Anionic Viscosity: 200–500 cP for 1% solution Density: 500–700 kg/m ³	1.0% dosage is sufficient to erect low-rise buildings on treated soil	(Ma and Ma 2019)
CH	Crustacean shells	Cationic Insoluble in water, soluble in 1% acetic acid solution.	Strength improvement – soil stabilization	(Hataf et al. 2018; Shariatmadari et al. 2020)
Gellan gum	<i>Sphingomonas elodea</i> microbes	Anionic	Strength improvement – soil stabilization	(Chang et al. 2016)
GG	Endosperm of guar seed	Nonionic pH: 7.47 Viscosity: 12 cP for 0.25% solution	Clay liners, ground improvement for foundations slope stabilization	(El-Daw 1994; Caballero et al. 2016; Anandha Kumar and Sujatha 2021)
Starch	Plant extracts	Ionic nature Molecular weight depends on source and production methods	Soil strengthening, erosion reduction	(Khatami and O'Kelly 2013; Chang et al. 2020)
XG	Aerobic fermentation of sugars by the bacterium <i>Xanthomonas campestris</i>	Anionic pH: 8.2 Viscosity: 174 cP for 0.25% solution	Landfill liners, ground improvement for foundations	(Ayeldeen et al. 2017; Anandha Kumar and Sujatha 2021)
β-Glucan	<i>Saccharomyces cerevisiae</i> yeast	Anionic pH: 6.95 Insoluble in water	Landfill liners	(Chang and Cho 2014; Anandha Kumar and Sujatha 2021)
Agar	Cell walls of red algae	Non-ionic	Foundation applications	(Khatami and O'Kelly 2013; Fatehi et al. 2021)
Persian gum	Plant exudate	Anionic pH: 4.4 Viscosity: 48 cP for 1% solution.	Soil stabilizer and purifier	(Ghasemzadeh and Modiri 2020)
Casein	Dairy products	Anionic Insoluble in water	Soil stabilizer	(Fatehi et al. 2021)
Albumen	Egg shells	pH: 8–9.5 Hydrophilic	Strength improvement	(Zarei et al. 2022)

Note: CH chitosan, CMC carboxymethyl cellulose, GG guar gum, XG xanthan gum

2019; Ko and Kang 2020; Kwon et al. 2020; Devrani et al. 2021; Mahamaya et al. 2021). Ayeldeen et al. (2016) used modified starch in combination with XG and GG for improving the soil erosion resistance against various wind speeds. Wade et al. (2021) used GG and alginate to improve soil aggregation and crust integrity, as these polysaccharides strengthen the soil crust. As BBST increases soil cohesion, it can be employed to improve the stability of the soil slopes, and to prevent shallow landslides (Caballero et al. 2016; Acharya et al. 2017; Zhang et al. 2021; Che et al. 2022; Kar et al. 2022). GG slurry has been used to prevent the sidewall collapse during trench excavations (Aminpour and O’Kelly 2015). Ko and Kang (2020) demonstrated the potential application of BBST to resist levee breach, and they observed that the biopolymer-stabilized soil approach provided better flood protection compared to vegetated levees. Lee et al. (2019) used bottom ash along with XG to stabilize the road shoulder, with XG addition producing significant improvements in the subgrade strength. Aminpour and O’Kelly (2015) reported that biopolymers find various applications in dam construction and operation activities. Bouazza et al. (2009) recommended BBST for conditioning soil used as liners in waste and hazardous contaminant facilities, since the biopolymer additives were found to significantly reduce the soil permeability. Aminpour and O’Kelly (2015) and Anandha Kumar and Sujatha (2021) reported that BBST can modify the soil into a low permeability medium, thereby facilitating its use in barrier and clay-liner applications. Biopolymers can withstand environmental conditions of wetting–drying and freezing–thawing (Chang et al. 2015; Sujatha and Saisree 2019; Singh and Das 2020). Here, the soil–biopolymer interaction mimics adhesive action that helps to resist degradation of the treated soil, thereby achieving reduced volumetric change with water content variation (Chang et al. 2015; Sujatha and Saisree 2019). Khatami and O’Kelly (2018) added XG and diutan gum to superfine ground-granulated blast-furnace slag (GGBS) cement, and explored ways to increase the zeta potential of fine grout particles such that they remain in suspension for reasonable time periods to allow successful implementation of the grouting processes. The purpose of including these biopolymers in the cement grout was to fortify the grout composition to have a sedimentation/bleeding resistance.

Changes in Soil Plasticity for Biopolymer Addition

Biopolymers modify the particle aggregation and water absorption in treated soils making investigations of Atterberg’s (consistency) limits and other indices for the treated soil a necessity in advocating BBST considering various geotechnical and geoenvironmental applications. Hydrophilic biopolymers, including XG, GG, cellulose, sodium alginate (SA), agar, carrageenan gum (CG), and β -glucan, generally cause an increase in the liquid limit (LL) water content. For instance, Nugent et al. (2009) investigated the effect of XG and GG additions on kaolinite clay and found that LL tends to increase with biopolymer dosage on account of the increase in viscosity of the pore fluid, especially at higher concentration. Whereas at a lower concentration, these authors observed that the biopolymers tended to aggregate the

soil particles causing a small decrease in LL. Furthermore, GG addition resulted in a higher LL than XG because of the abundant network of hydrogen bonding between the GG and kaolinite particles. Kwon et al. (2019) and Sujatha et al. (2021) also observed a similar increase in LL for XG-treated soft marine clay and clayey sand, respectively. These authors attributed the increase in LL to hydrogel formation in the soil–biopolymer matrix and to electrical interaction between the XG and clay component in the soil. Cabalar et al. (2018) recorded similar results of increasing LL with XG addition. These authors also observed a marginal change in linear shrinkage on addition of XG (attributed to retardation in the rate of water evaporation), although the swell ratio was found to increase (from approx. 1.5% to 8.5%) with increasing XG dosage. This retardation in the evaporation rate in the presence of XG may influence soil shrinkage and control differential movement, potentially limiting damage to infrastructure built on clay soil deposits (Cabalar et al. 2018).

Zhanbo et al. (2020) investigated the effect of six different biopolymers (i.e., XG, GG, SA, agar, locust bean gum, and CG) on kaolinite. The observations from their study showed that CG, locust bean gum, and GG caused a greater increase in LL compared to agar, XG, and SA. For non-viscous biopolymers, like β -glucan, a marginal increase in LL was observed for increasing biopolymer dosage, although there was a slight decrease in plasticity index (Chang and Cho 2014; Kumara and Sujatha 2020). The decrease in plasticity index occurs because the thread-like fibers (formed on β -glucan addition) rendered the soil matrix stiffer. Similar behavior was also observed by Abd et al. (2022) on addition of cellulose (low viscous biopolymer) to clay soil, with the LL increasing appreciably (from 39% to 72%), but the plasticity index decreasing marginally from 25% to 19%.

The above literature shows that viscosity plays an important role in modifying the plastic nature of biopolymer-treated soil. Judge et al. (2022) reported that the polarity and viscosity of the biopolymers modify the LL of soft clay soils. The size of the molecules and their shapes control the viscosity, while polarity dictates the interaction between the charged soil particles and biopolymers. Judge et al. (2022) noted that the addition of GG and CG, both of which have high viscosity, caused a significant increase in LL for the biopolymer-treated soils.

Biopolymers for Strength Improvement

Various biopolymers, including XG, GG, gellan gum, SA, β -glucan, CH, casein, modified starch, and agar, have been used, as additives, to improve the strength of different soil types; that is, sand, silt, clay, and organic soils. Ivanov and Chu (2008) reported the mechanisms that improve the strength, and reduce the permeability, of biopolymer-treated soil, as being biocementation and bioclogging, respectively. BBST, with the biopolymer molecules having electrical charge and a high specific surface area (SSA), creates a soil–biopolymer matrix that mobilizes higher strength compared to the untreated soil (Chang et al. 2016).

For coarse-grained soil, BBST improves the interparticle cohesion (and soil friction angle) through particle aggregation. The friction angle increases on account

of the larger particle contact area facilitated by biopolymer gel formation in the pore-void spaces (Chang et al. 2016). On drying, the gel stiffens and provides greater strength (than when wet). For clay soil, or soil with clay in its matrix, the biopolymer and clay particles bond with each other, owing to their charged surfaces, thereby forming a coating with enhanced interparticle contact (Chang and Cho 2019).

Cabalar et al. (2018) investigated the effect of XG dosage on clay soil for different curing periods. The fall cone and vane-shear undrained shear strengths of the treated soil was found to increase for increasing XG dosage and longer curing period. Here, the high SSA of XG enables better interaction between the soil matrix and biopolymer, thereby producing improved strength, although XG addition did not influence the soil stiffness, owing to electrostatic repulsion between the XG strands and clay particles. Chen et al. (2019) employed XG to improve the shear strength of sand, and observed that drying had a considerable effect on the mobilized strength. Also, their study showed that moisture content was the determining factor for soil strengthening, i.e., at higher water contents, the adhesive stress was low, whereas for the same dosage the adhesive stress nearly doubled on drying.

CH is also effective in improving the strength of clay soil through interparticle interaction that depends on the moisture content and curing period (Hataf et al. 2018). The cationic nature of CH enables electrical interactions to occur between the biopolymer and the diffuse double-layer of the clay particles, and thereby improves the interaction between the soil particles. Zinchenko et al. (2022) proposed the in-situ gelation of CH (cationic) and CMC (anionic) to improve the strengths of sand, clay, and sand–clay mixtures. This in-situ gelation technique offers a more flexible design for soil improvement. Clay treated with the poly-ions showed significant strength improvement, which was attributed to large ionic bonds formed between the clay particles and poly-ion solution, owing to the large SSA of the clay particles. However, for the sand, the strength decreased because of its small SSA and more porous structure.

Chang and Cho (2019) investigated the effect of gellan gum on the strength of clay, sand, and sand–clay mixtures through a program of direct shear and vane-shear strength tests. Gellan gum improved the strength of the selected soils for all dosages and water contents investigated. The shear strength of the gellan gum-treated sand was lower compared to gellan gum-treated soil containing clay, that is, the sand–clay mixtures. The increase in the cohesion and friction angle parameters was dependent on the gellan gum dosage, and was higher for the sand–clay mixtures. Also, for higher gellan gum dosage, the soil exhibited brittle and dilatative behaviors, which these authors attributed to the soil agglomeration effect. Smitha et al. (2021) used agar solution prepared at 100 °C to improve the strength of silty soil samples, employing 0.5%, 1%, and 2% agar dosage. The samples were cured at 30 °C for 0, 1, 3, 7, and 28 days, with the maximum strength gain observed for 2% agar at 28 day curing. As fitting parameters for the Mohr–Coulomb strength (failure) envelope, the deduced cohesion parameter was found to increase with agar dosage and for a longer curing period, but the deduced friction angle did not show such a characteristic response, instead tending to decrease for a longer curing period. Wen et al. (2019) investigated the strength improvement of sand impregnated by a

hydrogel of SA, with the unconfined compressive strength (UCS) of the sand treated with 0.4% SA dosage improved to 430 kPa.

Chang and Cho (2012) examined the strength behavior of β -glucan treated Korean residual soil. These authors found that the UCS of the treated soil increased by nearly 200%, which they explained by the soil particles accumulating around bundles of β -glucan (on account of the negative charge of the residual soil), thereby improving the ionic bond between the β -glucan chains and the soil particles. These authors also investigated the UCS of the β -glucan treated residual soil for different temperatures of 20 °C, 60 °C, and 100 °C, with the UCS mobilized at 60 °C substantially improved for the maximum dosage of 4.92 g/kg (of β -glucan to soil) investigated. Kumara and Sujatha (2020) also found β -glucan effective in improving the strength of lean clay, with both the deduced cohesion and friction angle parameters increasing for greater dosage and longer curing period, i.e., from a UCS of 62 kPa for the unamended soil, to 180 and 610 kPa for 2% β -glucan dosage, investigating one-day and 28 day curing, respectively. These authors attributed the strength increase to ionic bond formation because of the charged surfaces in clay and β -glucan.

Ayeldeen et al. (2017) used XG and GG to improve the mechanical behavior of collapsible soil. The shear strength parameters for dry and soaked conditions were measured immediately after adding the biopolymers to the soil, and then for seven-day curing. Both biopolymers were found to appreciably reduce the collapse potential of the treated soil, with GG performing significantly better than XG for both the immediate and seven-day curing scenarios investigated. These authors credited the better performance of GG to its higher molecular weight, which enabled the biopolymer to cross-link between the soil particles, and also reduced the soil permeability. Numerical analysis performed as part of their study showed that biopolymer addition improved the bearing capacity, and additionally reduces the settlement and effect of saturation for the studied collapsible soil. Dehghan et al. (2019) also investigated XG and GG to stabilize collapsible soil and to reduce the collapse potential. Both of these biopolymers improved the soil strength for all dosages and curing periods investigated. Kumar and Sujatha (2021) investigated XG and GG to improve the strength of clayey sand and found that XG showed better performance than GG, although both biopolymers were found to appreciably improve the soil strength. These authors concluded that the charged nature of XG enabled ionic bond formation that was responsible for the superior behavior of the XG additive.

Erosion Resistance of Biopolymer-Treated Soils

BBST is a promising technique for improving the mechanical stability of soil prone to erosion, and it can also promote an improvement in soil health (Wade et al. 2021). For example, biopolymer treatment improves both vegetation germination and soil water-retention characteristics against evaporation (Chang et al. 2015).

Kavazanjian et al. (2009) investigated spraying of 0.5–2 g/l XG solutions on the soil surface and performed simulated wind-erosion tests, observing a reduction in

soil loss to as little as 1 wt%. Ayeldeen et al. (2016) compared different polysaccharides, including XG, GG, CG, and modified starch, for improving the soil erosion resistance, and found that GG mobilized the maximum shear strength against wind erosion, and thereby experienced the least erosion (loss in soil mass). Ham et al. (2018) investigated the choice of an in-situ microbial biopolymer formation technique to deter soil susceptibility to erosion. These authors suggested increasing the sucrose concentration in the nutrient solution, and/or the volume of nutrient solution being injected, to augment the in-situ microbial biopolymer formation.

Joga and Varaprasad (2020) reported that XG effectively reduced the dispersive potential at 0.5–2% dosage by reducing the percentage of sodium in the dispersive soil investigated. They observed that 1% XG was the optimum dosage for mobilizing the maximum erosion resistance, which was attributed to gel plug formation and ionic bonds holding the soil particles together, thereby preventing them from being lifting off the soil surface. Wade et al. (2021) found an improvement in wind erosion resistance achieved by spray application of GG and SA solution. By allowing better diffusion, as SA (of low viscosity) was employed at a higher dosage of 5 g/l, this improved the aggregate size and UCS of the treated soil. Their study also showed that alginate produced stronger clods, resulting in stronger soil crust formation. Devrani et al. (2021) investigated various XG solution dosages, as well as the microbial-induced calcium carbonate precipitation (MICP) technique, to assess their efficiency in controlling the erosion of Rajasthan desert sand. [See, for example, Shahrokhi-Shahraki et al. (2015), Zomorodian et al. (2019) and Choi et al. (2020) for elaboration on the MICP technique] Both methods were found to significantly reduce the soil erosion (mass loss). For instance, the 0.25% XG-treated sand sample (with soil crust thickness of 2 cm) was able to resist a maximum wind velocity of 45 km/h. Shabani et al. (2022) investigated the biopolymer serish, a polysaccharide extracted from the root of the Eremuurs plant, for improving the strength and erosion resistance of dune sand, with, for instance, 2% serish-treated sand experiencing a soil mass loss of <1.21% when exposed to a wind speed of 25.2 km/h.

Orts et al. (2000) examined different biopolymers, including polyacrylamide (PAM), cellulose xanthate, starch xanthate, acid-hydrolyzed cellulose microfibrils, and CH, for reducing soil loss during irrigation. Their investigations comprised laboratory tests on a furrow arrangement for clay loam soil, and also field tests on furrows. Of the studied biopolymers, PAM was found the most efficient additive in resisting soil erosion during irrigation, with cellulose xanthate, starch xanthate, and acid-hydrolyzed cellulose microfibrils also producing good results. Based on their findings, Orts et al. (2000) recommended the use of BBST for various geotechnical engineering applications, including to mitigate erosion resistance of surficial soil layers at construction sites, and also for highway embankments. Through a series of laboratory simulations, Chang et al. (2015) demonstrated the potential of XG and β -glucan for improving the soil erosion resistance to heavy rainfall, and to deter desertification by promoting the growth of vegetative cover. Their study also underlined the capacity of BBST to improve interparticle cohesion and the soil's water-retention capacity, owing to hydrogel formation. Reddy et al. (2018)

investigated the stabilization of red mud (a dispersive industrial byproduct) using XG and GG additives. Their results showed that, whereas XG was not effective, 2–3% GG was found successful in arresting the dispersion phenomenon. Ko and Kang (2018, 2020) explored the possibility of reinforcing a levee slope employing a biopolymer additive, and found that the erosion resistance of the biopolymer-treated slope was 50% greater compared to the untreated slope. Their study also demonstrated that the breach mechanism for untreated and biopolymer-treated slopes was different, with delayed/inhibited initial erosion resulting in the water level reduction occurring at a slower rate for the biopolymer-treated levee. Mahamaya et al. (2021) investigated XG, GG, and CMC additives for improving the erosion resistance of two different dispersive materials, i.e., fly ash and organic silt, against both water- and wind-induced erosion. Their study reported that all three biopolymers produced appreciable improvements against water-induced erosion, as evaluated from pinhole and dispersion tests, with CMC showing superior performance compared to XG and GG. CMC also produced higher resistance to wind erosion, based on the reduced loss in soil mass for treated samples, evaluated for various wind velocities in wind tunnel experiments. These biopolymers coated the soil grains and bridged the pore-void spaces, either by gel-plugs or gel-threads, causing an increase in interparticle cohesion, thereby resulting in improved erosion resistance (Chang and Cho 2012; Ham et al. 2018). Greater erosion resistance can also be attributed to reduced seepage flow, owing to clogging of the pore spaces by gel-plugs.

The rheology of biopolymer-treated soil is distinct from the untreated soil, and is characterized by the absorption of moisture by the biopolymers due to their hydrophilic nature, plugging of the pore-void spaces, reduced seepage flow, and the hydrophobic behavior of the coated soil particles that cause high run-off (Chang et al. 2015). Furthermore, on drying, the hydrogel attaches itself to the soil particles, thereby forming a surface coating. The dehydrated soil matrix is stiff and resists volume reduction, thereby maintaining pores that are more open. This results in higher porosity, allowing more flow paths or easier root penetration to occur. For heavy rainfall, the hydrophilic nature of the biopolymers enables them to absorb more moisture, increasing their size, and thereby to fill (plug) the pore-void spaces and consequently reduce the infiltration by the bioclogging mechanism (Chang et al. 2015). Figure 1 presents the schematic process of improved resistance achieved for biopolymer-treated soil against water- and wind-induced erosion.

Biopolymer Applications in Contaminant Barriers and Clay Liners

Permeability is a critical factor to consider in selecting soil for use as a liner or contaminant barrier. Here, biopolymers can be employed to form gel-plugs that clog the soil's pore voids, thereby causing an exponential reduction in soil permeability (Bouazza et al. 2009; Wiszniewski et al. 2018; Sujatha and Saisree 2019; Anandha Kumar and Sujatha 2021). Also, with further moisture ingress, the hydrogel expands in volume resulting in filling of the pore voids. This characteristic makes

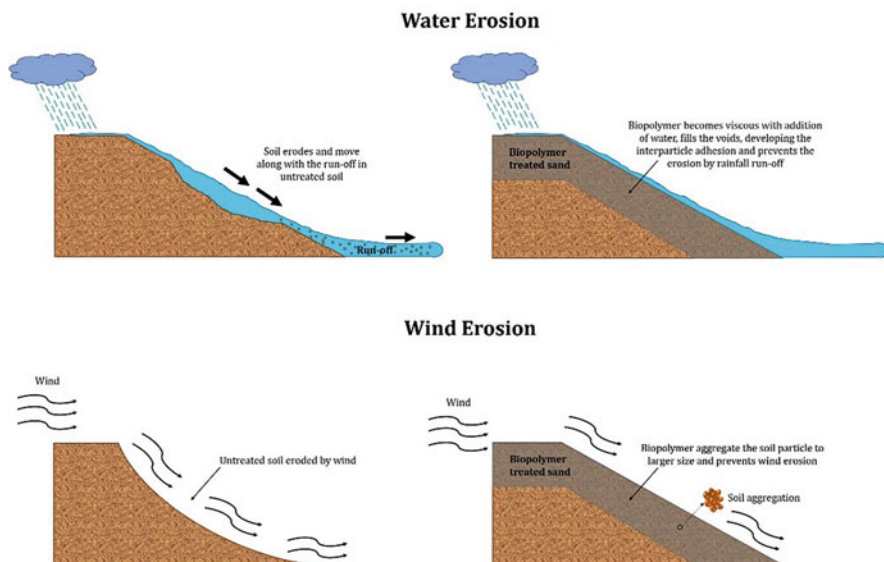


Fig. 1 Schematic representation of improved resistance achieved for biopolymer-treated soil against water- and wind-induced erosion

biopolymer-treated soil suitable for the construction of seepage barriers and slurry walls (Chang et al. 2016).

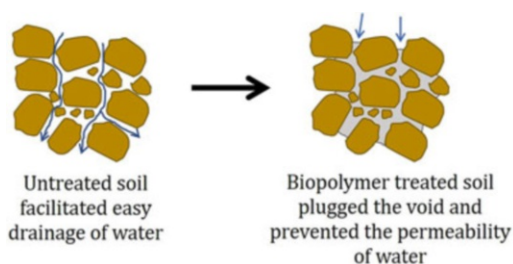
Various factors (e.g., the fines content and type of clay mineral in the soil, the biopolymer type and concentration, temperature, and aging (Choi et al. 2020)) affect the permeability of biopolymer-treated soil. Khachatoorian et al. (2003) investigated XG, GG, CH, polyglutamic acid, and polyhydroxy butyrate biopolymers for reducing soil permeability, and observed that they all produced substantial reductions in permeability. Over an 11 day curing period investigated, polyhydroxy butyrate achieved the maximum permeability reduction (of the order of 10^9 fold), while CH and polyglutamic acid biopolymers produced 10^6 fold reductions. Similarly, Bouazza et al. (2009) observed that XG, GG, and SA effectively reduced the permeability of silty sand, with, for instance, a 0.5% SA dosage reducing its permeability to (of the order of) 10^{-12} m/s for 70 day curing. Their study concluded that BBST can transform silty sand for use as a temporary seepage barrier material. Chang et al. (2016) investigated saturated gellan gum-treated soil, and found that it also exhibited a decrease in permeability due to the pore-plugging effect. Their study showed that 2% gellan gum additive produced a five order of magnitude reduction in permeability.

Ayeldeen et al. (2017) observed that XG, GG, and modified starch substantially reduced the permeability of treated sand and silt. A similar effect on the permeability of XG-treated sand was reported by Cabalar et al. (2018). For example, the permeability of 0.5% XG-treated sand showed a decrease from (of the order of) 10^{-6} to 10^{-11} m/s, and on increasing to 2% XG dosage, the permeability was reduced further (to 10^{-12} m/s). XG and GG were used to treat low-plasticity silty clay, and the results showed that the permeability reduction was dependent on the packing density,

biopolymer dosage, and curing period (Dehghan et al. 2019). Owing to its anionic nature, XG was also found to modify the permeability of soft marine clay (Kwon et al. 2019). The feasibility of using ϵ -polylysine, a cationic polymer, to reduce the permeability of high-plasticity clay was investigated by Kwon et al. (2019). Their results showed that although there was a permeability reduction for the clay, it was less than that achieved for XG treatment. Singh and Das (2020) reported a sharp reduction in the permeability of XG-treated expansive silt for various dosages and applied consolidation pressures investigated. Sujatha and Saisree (2019) observed that GG additive slightly reduced the permeability of silty clay – after full saturation for 6 h, the permeability had decreased from (of the order of) 10^{-6} to 10^{-7} m/s for 2% GG dosage. Biju and Arnepalli (2020) noted that for 0.5–3% dosages, both XG and GG increased the permeability of sand–bentonite mixtures. These authors concluded that the aggregation of the clay particles promoted by the biopolymer additives widened the pore void spaces, thereby causing the observed increase in permeability. Their study emphasizes the importance of considering both the type of soil and biopolymer when seeking to modify the soil permeability by BBST.

Using bacterial biopolymers produced from indigenous bacterial species, Kim et al. (2019) investigated the bioclogging mechanism in coarse sands to arrest leakage in waterfront structures. Their study showed that the soil's particle-size distribution, the choice of buffer, and the applied backpressure contributed to the change in permeability, with, for instance, greater permeability reduction achieved for fine sand (than coarse sand) due to its smaller pore-void spaces. SA effectively reduced the permeability of poorly graded sand (Wen et al. 2019), with, for example, the permeability decreasing from the order of 10^{-5} (for the untreated sand) to 10^{-7} m/s for the 0.4% SA-treated sand. Anandha Kumar and Sujatha (2021) evaluated the choice of XG, GG, and β -glucan for modifying clayey sand as a contaminant barrier/liner material. Their study also reported on the influence of the biopolymer type, its dosage, and the curing period on the change in permeability, with biopolymer-treated soil undergoing a remarkable permeability reduction of 3–5 orders of magnitude relative to the permeability of the untreated soil. With aging, a slight increase in permeability was observed owing to the hydrogel shrinkage on dehydration, but in all cases investigated the permeability remained much lower than that of the untreated soil. These authors argued that the slight increase in permeability would not negate the choice of these biopolymers for modifying soil into impermeable barriers for landfills or contamination facilities. They also studied the leachate-attenuation capacity of the selected biopolymers, and observed that XG showed the best performance in absorbing almost all heavy metals. Anandha Kumar and Sujatha (2022) compared the performance of calcium bentonite-modified silty clay and XG-treated silty clay for use as clay liner material and found that the XG-treated silty clay had higher strength and lower permeability when compared to the calcium bentonite modified soil. For instance, the 1% XG-treated soil had permeability nearly 30% lower than the calcium bentonite-modified soil. These authors advocated the choice of XG additive to modify soil for construction of clay liners on the merits of its environmentally friendly nature, negative carbon production, and non-polluting behavior. The bioclogging mechanism responsible for reducing the permeability of the biopolymer-treated soil is shown in Fig. 2.

Fig. 2 Bioclogging mechanism for biopolymer-treated soil



Challenges and Future Research Needs

To date, only a few studies have investigated the economic aspect and environmental impact of BBST application on a large scale. Biopolymers are presently less competitive economically compared to conventional soil binders, like cement and lime. However, since biopolymers also find various applications in other fields (e.g., the food, textile, cosmetic, pharmaceutical, and medical industries), there is scope for their mass production, which in-turn can bring about a reduction in their cost. For instance, in the 1960s, the cost of XG was 30,000 US\$/ton, but this had reduced to 1500–4000 US\$/ton by 2020, providing an expectation of its large-scale geotechnical/geoenvironmental engineering application in the near future. Possible environmental impacts of BBST application that require investigation include the effects of the consequent elevated soil temperature and change in soil pH, and that biodegradability may affect its durability. For instance, the way that biopolymers are degraded when used in large volume raises concerns about potential contamination of the terrestrial environment. Sensitivity to water is another major consideration; strength loss and apparent swell/shrink behavior are the drawbacks (Fatehi et al. 2021).

Limited field studies have been performed to evaluate BBST, or to investigate the changing characteristics of biopolymer-treated soil under real environmental conditions, for example, varying temperature and pH, wetting–drying cycles, and micro-organism and (for surface-treated soils) ultraviolet-radiation attacks. Chang et al. (2020) reported a trial field study investigating XG, casein, and XG–starch combination for stabilizing, and promoting vegetative growth on, the side slopes of an embankment at a pilot construction site in Seosan, Korea. On-site observations after 100 days showed increased shear strength and vegetation density.

Biopolymer durability in long-term field applications requires more investigation to understand how different biopolymer types behave under various environmental and loading conditions. For instance, since many biopolymers are soluble in water, exposure to water would likely affect the BBST performance during wetting–drying cycles. Grouting/injection of biopolymers in soil also remains a practical drawback (Khatami and O’Kelly 2022), with field applications of BBST limited by the development of necessary equipment that is compatible with the rheology of the biopolymers. Due to increased viscosity, injection of biopolymer-added grout

typically requires higher injection pressures that depend on the biopolymer concentration/grout viscosity (Khatami and O’Kelly 2022).

Summary and Conclusions

The geotechnical engineering sector consumes huge amounts of chemical additives (conventionally cement and lime), as binders, most of which are not ecofriendly. Of the various biological methods, BBST represents a promising green and sustainable alternative for soil strengthening (i.e., biocementation) and in reducing the soil permeability (i.e., bioclogging). This chapter elucidated the potentials of BBST for various geotechnical and geoenvironmental engineering applications, including the stabilization of weak soils, slopes, and excavations, for modifying marginal soil as contaminant barrier material, and in providing improved resistance to combat erosion of surficial soil layers, to name a few. For instance, their tendency to improve the interparticle cohesion potentially makes BBST particularly suitable to resist water- and wind-induced surface erosion. Furthermore, BBST may improve soil health, that is, by increasing the soil’s organic matter content and water-retention capacity, thereby promoting the growth of vegetative cover.

On the technical side, various factors must be carefully considered to achieve optimum BBST results, including the type of soil (i.e., its gradation, fines content, and the clay mineral type present), the biopolymer type and dosage, the moisture condition (saturation level), and curing period. Biopolymers modify the plasticity characteristics of fine-grained soil owing to their hydrophilic nature and, in the case of gelling biopolymers, the viscosity of the pore-fluid. Biopolymers also reduce the permeability of the treated soils, as the hydrogels tend to swell when in contact with water, thereby clogging the pore-void spaces.

The limited field implementation trials performed, to date, have reported enhanced engineering performance achieved by BBST. However, more studies are needed on the technical, environmental, and economic aspects of BBST application on a large scale. Additional research by way of comprehensive field trials are still required to translate and upscale the promising experimental outcomes to geotechnical and geoenvironmental engineering practice, investigating, for instance, the changing characteristics of biopolymer-treated soil for real environmental and loading conditions (e.g., involving wetting–drying cycles and microorganism attack). Grouting/injection of biopolymers also remains a practical issue, with the development of field equipment that is compatible with the biopolymer rheology necessary.

Cross-References

- [Biopolymers as Engineering Materials](#)
- [Mechanical Properties of Biopolymers](#)

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Abstract

The search for eco-friendly and renewable resources endowed with high stability and low toxicity has directed researchers around the globe to focus their attention on biomaterials, namely, biopolymers, which reduce the pollution caused by the rise in synthetic material-mediated pollution. As investment and research into biopolymers increases, several conventional material utilities are reduced in various optoelectrical, biological, and technological applications. Biopolymers in conjunction with nanotechnology have opened up novel and extremely useful arenas for their applications, namely, in the biomedical, energy sector, etc. This chapter is envisioned to outline the significant status of biopolymers as a

P. S. Shasiya · K. Simi Pushpan · A. B. Nair (✉)
Department of Chemistry, Union Christian College, Aluva, Kerala, India
e-mail: ajaleshbair@uccollege.edu.in

promising engineering material employed in various fields. In addition, future challenges and perspectives are also discussed in detail.

Keywords

Biopolymers · Classification · Engineering materials · Biomedical applications · Cosmetics

Introduction

Biopolymers are polymers manufactured chemically from biological compounds or derived by biosynthesis. They have several pros over synthetic polymers, such as more complex, well-defined structure, renewability, and degradability (Cziple and Marques 2008; Augustine et al. 2013). The distinction between biomolecules and biopolymers is often misunderstood. Biomolecules like the polyamine acid family are found in living organisms, and natural organic substances are not engineering materials. Hence, these biogenic macromolecules are not biopolymeric materials. Biomolecules that can be biotechnologically further converted into raw ingredients for polymer production are an exception. Polysaccharides and some bio-based acids and vegetable oils that can be used directly as raw materials are examples of exceptions.

Degradability is ultimately determined by the chemical as well as physical microstructure of the polymer and is independent of the source of the raw components employed or the manufacturing process. A polymer substance satisfies at least one of the following criteria, according to the best current generic definition of biopolymers:

- It is made up (in part) of sustainable raw materials.
- It is biodegradable.

As a result, biopolymers do not have to be made entirely from renewable resources. Petrochemical raw ingredients such as polyvinyl alcohols, polycaprolactone, copolyester, and polyester amide can also be used to make biodegradable biopolymers.

Biopolymers derived from various sources have been studied for pharmacological and biological applications for many years. Currently, biopolymers are used as an efficient active ingredient of medication or various healthcare products on the market. Biopolymers attract attention owing to their various compositions, tunable physical characteristics, and plenty of interesting options. Furthermore, the comparatively cheap cost and sustainable nature allow such materials to be significantly interesting for expensive industries like pharmaceutical and biomedical (Niaounakis 2015).

The only polymer materials developed from renewable resources at the start of industrialization were cellulose derivatives and rubber. At that time, natural raw

materials were only available in sufficient quantities. With the successful advancement of the petrochemical industry, renewable resources such as polymer raw materials were suppressed in favor of petrochemical-based raw materials (Stevens 2004). In recent decades, the first biologically degradable polymer compounds, such as polyvinyl alcohols or polycaprolactone, were produced based on these petrochemical raw ingredients for specific niche applications (Imam et al. 1999). Even though petrochemical-based plastics offered unparalleled processing and use features, the reliance on limited foreign resources for raw ingredients and the unfavorable disposal properties prompted a shift in focus. Since the end of the 1980s, polymers' disposable and recycling ability received more attraction and a growing number of degradable polymers based mostly on renewable resources have been industrialized. Meanwhile, the anticipated scarcity of resources and steadily rising prices of petrochemical raw materials have turned the focus of attention to biopolymer materials. Compostability is no longer the most important factor; instead, the use of renewable, i.e., long-term available resources for manufacturing raw materials, has taken precedence.

Innovative biopolymers, particularly those based on starch and polyhydroxy alkanooates produced by fermentation, were first introduced to the market more than 30 years ago, i.e., in the early 1990s. Despite some euphoria and promising prognoses, these biologically degradable first-generation biopolymers were unable to establish themselves in the market, owing to their yet-to-be-tested material properties, unfavorable political and economic conditions, and a lack of political resolve among industry and policy decision-makers (Endres et al. 2007).

Of late, work has begun on the development and optimization of a wide variety of biopolymers as the regulatory framework has changed. In terms of manufacturing and use qualities, improved second-generation biopolymer materials are currently on the market and are substantially comparable to traditional bulk plastics. They are becoming a progressively exceptional utility as packaging components (Platt 2006). Biopolymeric materials are now subject to slight economic drawbacks, owing to their still limited production volume. Once disposal expenses are factored in and/or production levels are increased further, this will be offset. The second-generation biopolymers find their utility as sustainable materials. These second-generation biopolymers are now being used to make polymer materials for engineering applications, and they are gaining popularity as they make their way into other industries like automotive and textiles (Holm Vibeke et al. 2006).

The trend in third-generation biopolymer materials is toward resistance rather than degradability. Simultaneously, in light of the restricted availability of petrochemical raw materials, the utilization of renewable resources for material manufacturing is gaining traction. One factor to examine is the long-term availability of the raw material base. Beyond that, further technological features such as heat resistance, odor, brittle fracture behavior, color, UV stabilization, and long-term reliability will be prioritized in the development of these biopolymer materials. The invention, testing, and application of relevant biopolymer additives, as well as the optimization of biopolymer production and processing properties, will be hot subjects (Smith 2005) (Fig. 1).

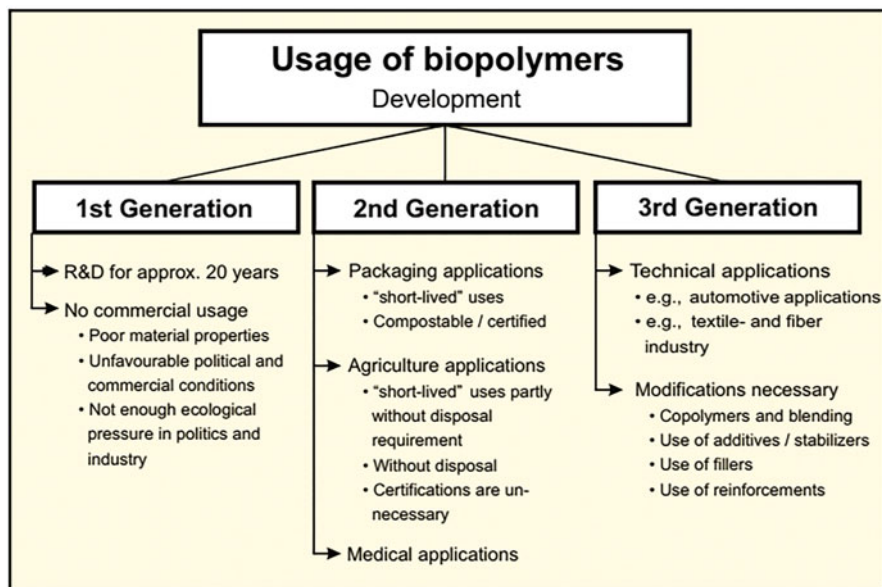


Fig. 1 Development of biopolymer (Endres 2011)

Rubber, linoleum, celluloid, and cellophane were among the first present-day biomaterials created from natural biopolymers. Cellulose is the source of the latter two and is the most abundant organic biopolymeric material. Petrochemical-based materials have largely supplanted human-made biopolymers that have attracted extraordinary interest from industry, the academic community, and policymakers (Baillie 2004) as a result of growing ecological concerns. Biopolymers have plenty of utility as packaging materials, medical field, food additives, cosmetics, adsorbents, textiles, sustainable plastic, biosensors, etc. Biopolymers are promising materials because of their abundance, biocompatibility, and unique qualities such as nontoxicity, among others. Biopolymers are being explored for use in a growing number of ways using nanosized reinforcements to improve their characteristics and practical uses (Bastioli 2005).

Classification

Biopolymers are giant macromolecules consisting of a large number of identical units (Guzman et al. 2011). In comparison to synthetic plastics, these polymers have been in the biosphere for billions of years. As compared to their synthetic analogs, biopolymers exist in an orderly fashion disintegrating long chains by various bioprocesses like metabolic degradation by enzymes and other environmental factors (Wang et al. 2015). The presence of the hydroxyl group causes the breakdown of biopolymer creating hydrophilic metabolites.

Biopolymers can be categorized in several different ways, namely, their origin, degradability, and polymer backbones. Biopolymers are separated into natural, synthetic, and microbial biopolymers based on their origin. Classification based on degradability has resulted in two groups, namely, biodegradable and non-biodegradable, while they are divided into many groups based on the polymeric backbone or functional group they possess. We can divide monomers into three categories based on their type: polysaccharide, protein, and nucleic acid. Another classification of biopolymers is based on their heat sensitivity, namely, elastomers, thermoplastics, and thermosets (Leja and Lewandowicz 2010) (Fig. 2).

Some of the classifications are

(a) Based on the origin:

- **Sugar-derived polymers:** In these types of biopolymers, sucrose is utilized as the basic production input. Lactic acid polymers are made from lactose from potatoes, maize, and other sources. Polyhydroxybutyrate is made using starch or sucrose as an input. Blowing, injection, vacuum shaping, and extrusion can all be used to make them. Lactic acid polymers (polylactides) derived from lactose obtained from potatoes, wheat, and sugar beet are found to be water-resistant (Monzer 2014).
- **Starch-derived polymers:** Polymers based on starch – starch is a natural polymer made up of glucose molecules. It is present in the tissues of plants. Wheat, tapioca, and potatoes all contain starch, which functions as a natural polymer. The substance is kept as one-way sugars in plant tissues. It is made up of glucose and is made by melting starch. Animal tissues do not contain this polymer. Tapioca, corn, wheat, and potatoes are examples of vegetables that contain them. Dextrins is a category of low-molecular-weight

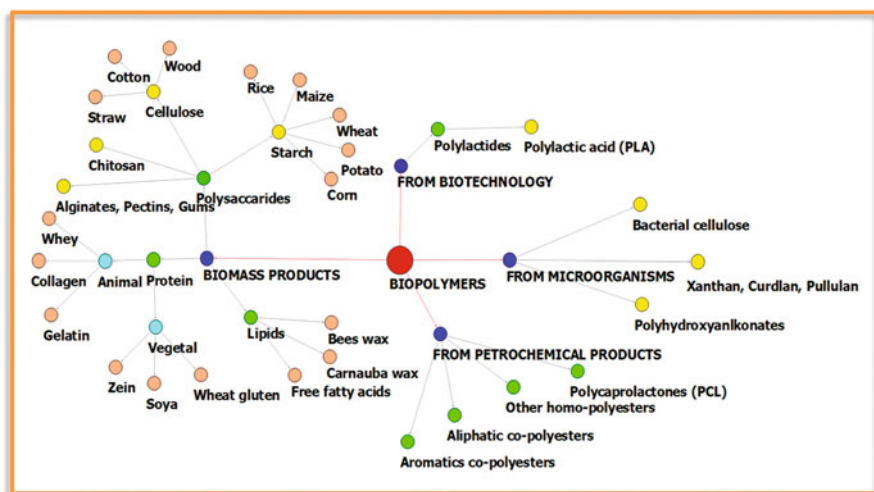


Fig. 2 Classification of biopolymer (Gheorghita et al. 2021)

carbohydrates generated by starch hydrolysis and enzymatically synthesized onto biopolymer carriers by immobilized *Enterococcus faecalis* Esawy dextranase (Esawy et al. 2016; Hashem et al. 2016).

- **Cellulose-based biopolymers:** This polymer is composed of glucose derived from natural sources such as cotton and is used for packaging. Consider cellophane. These are utilized in the packaging of cigarettes, CDs, and candy. The fundamental constituent of plant cellulose walls is this polymer, which is mainly composed of glucose. Cotton, wool, wheat, and corn are among the natural resources used to make them (Kaurov 2011).
- **Synthetic materials:** Synthetic materials derived from petroleum can be used to create biodegradable polymers. Biopolymers based on synthetic chemicals, such as aliphatic-aromatic copolyesters derived from petroleum, are also employed to make biodegradable polymers. Even though they are made of synthetic materials, they are entirely compostable and biodegradable (Kaurov 2011).

(b) Based on monomeric units:

- **Polysaccharides:** They are carbohydrate chains that are either branched or linear, for example, starch and cellulose.
- **Proteins:** Polymers built up of amino acids are known as proteins. Collagen, fibrin, and other proteins are examples.
- **Polynucleotides:** Nucleic acids are lengthy polymer chains made up of 13 monomeric units or more. DNA, RNA, and other similar molecules are examples (Nioounakis 2013).

The use of biopolymers is becoming increasingly popular among scientists. Because of their environmentally friendly nature, they can be used in a variety of settings. Because of their low cost, a variety of biopolymers are now utilized in industry. Because of their low cost and environmentally benign characteristics, these polymers will be used somewhat shortly. Blending and grafting are two techniques for increasing biopolymer characteristics. Biopolymers have been used to make a variety of composites and nanocomposites. Biopolymers are known to play an integral role in automotive, regenerative medicine, and packaging applications

The basic motive to promote the use of biopolymers is to replace many of the products we use every day that are manufactured from petroleum. This implies they will require qualities that are equal to, if not better than, the materials they are replacing to be suitable for the different applications they will be used in. Factors like the extent of polymerization and presence of additives and reinforcement agents and the quantity of these elements define the property and quality of the biopolymers. Lots of such efforts have been recorded by scientists around the world to study their thermophysical and mechanical properties (Augustine et al. 2013). Electro-active biopolymers derived from biological sources like starch, cellulose, chitosan, and pectin possessing electrical and ionic conductivity (EABP) are actively pursued to replace other synthetic nonrenewable materials (Niaounakis 2015).

Biopolymers as Engineering Materials

Biopolymers have various industrial applications in the area of food packaging, agriculture, medicine, and cosmetics (Fakirov and Bhattacharyya 2007). Some of them were found to be capable of replacing petroleum-based plastics and also fit for specific applications where plastic does not work, like artificial tissue development. The major requirement of such applications is a material with biodegradability and biocompatibility, and also sensitive to pH changes, as well as thermal and physico-chemical fluctuations (Imam et al. 1999).

When compared to synthetic polymers, biopolymers show poor chemical resistance, mechanical properties, and processability. Biopolymers reinforced with fillers called biopolymer composites were found more fit for specific uses owing to a drastic improvement in these properties. Of late, the demand for biopolymers surged, with the advent of novel and potential applications apart from being an integral part of being utilized in everyday life offering a sustainable substitute to plastic. Some of the major applications of biopolymers are summarized below.

Biopolymer-Based Antimicrobial Packaging

Environmental and microbial degradation of food products upon storage is a big challenge to the quality of food. The damage to food is due to the changes occurring in the food components and its exposure to processing conditions and environmental factors (Balasubramaniam and Chinnan 1997; Labuza and Breene 1989). Prevention of such damage from manufacturing time to consumption is the main objective of food packaging. The protective covering provided at the time of processing and storage prevents harmful deterioration of food and also enhances the food quality.

Packaging using raw materials of agricultural and marine origin is termed biopolymer-based packaging. They improve food safety as well as shelf life. Moreover, they effectively bring down the global carbon footprint arising from packaging systems specifically designed for food. The commonly used bio-based polymers for food packaging include polylactic acid, chitosan, cellulose, and starch. In addition, bio-based films and containers have the potential to replace oil-based packing materials providing enhanced technical properties and giving a sustainable and greener face (Vermeiren et al. 1999).

Polysaccharides are nontoxic biopolymers widely distributed in nature. Their selective permeability to CO_2 and O_2 retards the ripening of many vegetables and fruits by limiting respiration. Starch is an environmentally friendly, low-cost biopolymer with hydrophilic nature. Starch-based films show mediocre gas barrier properties while they lack better mechanical properties when compared to synthetic polymers. The addition of water acts as a plasticizer that imparts a thermoplastic behavior to starch. Starch-based coatings that contain potassium sorbate are reported to enhance the shelf life and reduce the microbial count (García et al. 1998)

Alginate is the salts of alginic acid and react with di- and trivalent cations which are utilized to form alginate films. Studies were carried out on the efficacy of a

calcium alginate film to prevent microorganism growth on beef and were found to have more acceptance from consumers (Williams et al. 1978)

Protein-based coatings on different vegetables and fruits obtained from milk, gelatin, wheat, etc., exhibit adequate barrier properties toward oxygen and carbon dioxide and are ineffective in the case of water. The WVP of the laminates was improved by incorporating corn zein but failed to improve the oxygen barrier property in contrast to SPI film (Anker et al. 1999).

In the beginning, the packaging mainly focused on the protection of food from physical damage, appearance, and durability of the package. Recently, the packaging technology stepped into the next level of development, giving more emphasis on safety traits with add-on antibacterial activity. The incorporation of antimicrobial agents into the packaging matrix is a big challenge as many of them studied are not suitable for food application. The selection of an antimicrobial agent is based both on its compatibility with the packaging material to be used and also on the heat liability of the antimicrobial compound during extrusion. Recent research has mainly focused on the development of new methods and their testing on model systems, but not in real food products. Technical feasibility, safety features of food antimicrobials, and consumer acceptance, along with the physiological, chemical, and microbial action of the packaging, must be the main focus of the food packing technology (Cha and Chinnan 2004).

Cosmetics

Cosmetic ingredients derived from living world (plants, animals, microbes, etc.) consist mainly of proteins. The cosmetic value of these proteins relies on their hydrophobicity and molecular backbone. Water-soluble hydrolysate of protein segregated from collagen is utilized in hair care products. Biopolymers like Gum Arabica are frequently used in the cosmetics industry owing to their nontoxic and nonallergenic properties. Furthermore, it stabilizes emulsions, aids in the rise of viscosity, gives the skin a smooth sensation, and produces a protective coating in creams and lotions apart from being utilized as a foam stabilizer.

Human and bovine collagen implants are exploited very well in cosmetic surgeries like dermatological healing, wrinkle removal, bone rebuilding, and dental and orthopedic surgeries. Due to the insolubility of collagen, only hydrolyzed collagen is employed in cosmetic formulations. Water-soluble small peptides and short polypeptides can be easily included in a variety of cosmetic compositions (Sionkowska et al. 2017). The lyophilization method is employed for the manufacture of collagen hydrogels and aerogels (Van Duong et al. 2018). The soluble collagen administered subcutaneously for healing dermatological defects is one of the most rewarding collagen applications boosting its popularity in cosmetic dermatology (Sionkowska et al. 2017). The types of chemicals that are added to a collagen filler formulation may vary depending on the requirements. Several research groups across the world are investigating collagen for improving cosmetic uses.

Keratin is the primary constituent of hair mainly utilized for conditioning though water-soluble hydrolyzed keratin is also employed in hair sprays and body creams (Jay et al. 1998). Crosslinkable keratin-based hydrogels are found to have enormous applications in mending wounds. This property of human hair-derived keratin-based hydrogels boosts its application in cosmetics as well. Apart from the gelling qualities, several natural polymers like cellulose gum find a key role as thickening agents. For instance, cellulose gum is a carboxymethyl cellulose derivative that is a water-soluble polymer and can be rheologically tuned by formulation specialists to have various physical properties like film formation, suspension, emulsion stabilization, etc. Cellulose gums have a thick, nonadherent surface whereas nanocellulose hydrogels, in particular, appear to have tremendous potential in energy storage, biomedical, culinary, cosmetic, and construction applications (Mezzenga et al. 2005; Coffey et al. 2006). Dextran-derived hydrogels have immense cosmetic potential (De Vuyst and Degeest 1999; Shahidi and Han 1993).

In many countries, notably in India (Biswas et al. 2001), placental protein is frequently employed in cosmetics and products used to cure an injury. Skin-whitening agents or UV-protective creams, antioxidants, or rejuvenation of skin help in maintaining youthful skin and thereby appear to have a good substitute for glutathione-based products. It bears exceptional antioxidative properties that decelerate the aging process by actively stimulating skin cell replenishment and actively by virtue of the natural components present in them and suppressing melanin production (Togashi et al. 2002; Olaiwan et al. 2010).

The aesthetics, biodegradability, and physiological benefits of the products are improved immensely by employing biopolymers as cosmeceuticals and additives. Swelling ability and mechanical strength are two important characteristics of hydrogel networks for cosmetics applications. The biodegradability of these biopolymers is an additional advantage. The advent of the twentieth century saw the boom of the polysaccharide industry followed by hydrocolloids based on pectins, chitosan, xanthan gum, alginates, etc. Polymers based on polysaccharides imparted water solubility and are functional food and cosmetic additives (Manjanna et al. 2010; Timerbaeva et al. 2007) whereas hydrocolloids derived from plants and microbes were utilized to give cosmetic and toiletry compositions a variety of distinct properties, namely, enhancement of water resistance, gloss, adhesion, and flexibility. The cosmetic industry relies heavily upon both animals and plants to provide proteinaceous components. Fungi and algae-derived proteins are gaining more popularity as protein sources with positive effects as topical agents (Secchi 2008). Protein's capacity to hold water to the peripheral skin layer is essential for the well-being of skin and hair. Simple proteins are utilized to make the majority of aesthetic protein derivatives, as opposed to conjugated proteins. The amphoteric and buffering capabilities of proteins are known to protect hair fibers (Chvapli and Eckmayer 1985). Chvapli et al. (Montecucco and Molgó 2005) explored the significance of proteins, especially collagen in cosmetics, with a specific focus on its use in conjunction with detergents. The authors addressed a number of misconceptions about collagen's mechanism of action, as well as potential hazards if collagen-containing formulations are not administered on intact skin.

The replacement of synthetic analogs with biodegradable polymers by the researchers was mainly due to economic, environmental, and technological reasons. Producing renewable polymers from mature plants is a daunting challenge that includes successful collaborations between many sectors. Crop plant advantages will improve with a greater understanding of their chemical, biological, and physical activity. The success of biopolymer utilization is linked to various fields spanning science and technology streams, such as biotechnology, chemical engineering, genetics, and novel experiments, including extraction of efficient polymer and monomer extraction from plants and its amalgamation into biorefining facilities. Biopolymer's success will be aided by advancements in all of these sectors. Polymer chemistry, in particular, will play a key part in the economical and energy-saving translation of building blocks into polymers, as well as the alteration of the extracted polymers for use in a variety of products (Augustine et al. 2013).

In Geotechnical Engineering

In geotechnical engineering, improving soil qualities has been a long-standing effort and progress. Researchers have discovered several soil improvement techniques like reinforcement, stabilization, grouting, etc., of which some are found to have harmful effects on the environment. As a result, we have to take corrective action with environmentally friendly technology. To reduce carbon emissions in the cement manufacturing process, bio-mediated geochemical soil improvement strategies are devised (Cole et al. 2012; Mitchell and Santamarina 2005; Ringelberg et al. 2014). Alternatives to standard soil treatment and enhancement approaches include greener materials (Tran et al. 2019; Jeong et al. 2019; Lee et al. 2019) and procedures (Qabany and Soga 2013; Chang et al. 2015; Lin et al. 2016) (i.e., mechanical improvement and chemical treatment).

The bacterium *Xanthomonas campestris* produces an anionic polysaccharide called xanthan gum (Chang et al. 2016). Xanthan gum is utilized to decrease the porosity of sandy soils by stuffing their pores (Gioia and Ciriello 2006) and improving water retention and preventing soil erosion. In geotechnical engineering (Brune et al. 2010), reduced mechanical, chemical, and structural characteristics, as well as less biodegradation stability, are the main drawbacks of employing biopolymers in tissue engineering. As a result, further research is needed to change biopolymers so that new adaptable materials can be developed that are better suited for tissue engineering applications.

Polyacrylamide (PAM) is an effective water-soluble low-cost polymer widely utilized for water treatment, enhanced oil recovery (EOR), and soil improvement effects (Flanagan et al. 2002; Jung et al. 2013; Xiong et al. 2018). Partially hydrolyzed PAM containing 0.5 wt% NaOH performed better than a polymer flood when tested for EOR (Jung et al. 2013). Recent research has demonstrated that biopolymers can be utilized to improve and stabilize soil and that they offer a number of benefits. Adding biopolymers to soils has been shown in several studies to improve soil strength.

Cyamopsis tetragonoloba seeds yield a polysaccharide, guar gum, on extraction (Smitha and Sachan 2016; Chudzikowski 1971). Addition of guar gum to silt increased permeability, sand cohesiveness, and unlike other biopolymers like modified starch and xanthan gum. Over the ages, soil improvement strategies have been extensively researched and improved. The simplest and most straightforward approach for improving soil is to add cement to it. However, due to serious environmental concerns, the need for environmentally friendly construction technologies is growing. Adding biopolymers to soils has been shown in several studies to improve soil strength. Biopolymers interact with soils depending on the temperature, type, ratio, and response to the water offering several benefits for the stabilization of soil. Biopolymers deployed are considered to be in sync with the geographical characteristics in near future.

Civil Engineering Applications

Biopolymers enjoy a niche stature in civil engineering practices with substantial turnover in sales. The world's urban center population is expected to grow from 3.4 to 6.4 billion people between 2009 and 2050. To handle the growing population of people in the world's big cities, the construction sector is expected to increase at an exponential rate (Daramola and Ibem 2010). Seto et al. (2012) calculated that by 2030 there will be civil engineering structures based on biopolymers (Ashby 2015). Soil incorporated with biopolymers lowers the water conductivity while increasing absolute strength (Kavazanjian Jr. et al. 2009; Nugent et al. 2010). Biopolymers have a tendency to plug the holes and are used for biodegradable borehole drilling (DeJong et al. 2013). Biopolymers can be used to construct permeable reactive barriers for groundwater correction as temporary excavation support fluids. When sand is treated with biopolymer slurry, the permeability of the sand can be reduced in as little as 2 weeks. The hydraulic conductivity of sand is found to decrease while extracellular polymers are added to create hydraulic barriers (Khachatourian et al. 2003; Reddi and Bonala 1998).

Microbial polysaccharides can be employed as cement admixtures. Temporary structures are made of biodegradable microbial bioplastics. Biotechnologically produced bioagents like soil microbes or active cultures of native microorganisms can be employed in construction. In the building industry, biotechnology is used for soil bio-clogging, bio-desaturation, bio-cementation, and bio-aggregation. Biotechnologically produced construction materials and processes are superior to their traditional counterparts and if utilized to their full potential they might yield significant economic and environmental benefits (Çabalar and Çanakci 2005).

Use of lignosulfonate in construction is bound to decrease mainly due to lack of start material and shift in technology of modern times. Polymer composites containing biopolymers like starch, cellulose, or other synthetic polymers were being used in drilling oil well. Starch and cellulose-derived compounds rule the construction scenario as newer and advanced technological advancements were utilized to make it industrially viable and profitable business as it can be tuned

accordingly by chemical blending and introducing additives or modifiers. Microbial biopolymers offer extensive potential especially in providing sheer thinning rheological effect compared to synthetic and other biopolymeric competitors in this arena. Novel industrial products with superior potential with high-temperature tolerance were produced by mutant or novel species of microorganisms. Even though it is an expensive process, the advantages it imparts make it highly sought after despite the costs incurred. Moreover, in case of microbial biopolymers, existing microbes improve capacity to develop more fruitful organisms that ultimately advance the production of more similar microbes that make better nutrients, thereby improving the economics in the long run. Researchers are currently interested in finding new technological innovations to expand their production and utility, which already is superior to synthetic polymers.

Farm products are not damaged by biopolymers and are superior to petroleum-based polymers. Biomass and agricultural waste recycling boost environmental conservation, ecological equilibrium, and environmental cleaning (Gopalakrishnan et al. 2013; Gopalakrishnan et al. 2012; Hottle et al. 2013). The perceived advantage of biological polymers has been found to be appealing to the consumers as they are environmentally benign and the previous experiences with natural components with synthetic polymers have resulted in potential health hazards over the years due to toxicity associated with the majority of the synthetic analogs.

Agriculture

The financial damage in agriculture and food production is attributed mainly to pathogens and pests affecting the crop yields. Hence, ensuring excellent seed quality and their protection from disease-causing agents using various chemical agents has been a norm. Since the 1930s, all existing classes of synthetic polymers have found utility in various aspects of agriculture in controlling release of nutrients and pesticides, fumigation, conditioning of soil, etc. The advent of biopolymers allowed added excellent features like biodegradability and circular economy into the field of agriculture, which can convert the polymers into soil-enriching raw materials.

Agriculture is interested in investing in biopolymers due to scientific and commercial motives. Seed coating done by biopolymers offers stabilization, protection, enhancement of yield, and growth regulation of plants. Biodegradability and sufficient action lifetime of starch-based polymers make them attractive to be put into agricultural use, especially as nutrients and insecticides incorporated as controlled release agents. Its ability to disseminate, dissolve, and encapsulate the active ingredient helps the polymer matrix or coating as an attractive candidate in agriculture. It can also exist as a part of a macromolecular scaffold or as a peripheral substitution of a pendant. Chitin, alginic acid, cellulose, lignin, and starch can function as biopolymers commonly employed in controlled release systems (Prasad et al. 2007).

Carbohydrate-derivatized biopolymers, owing to their biocompatible, atoxic nature, are definitely a greener substitute for potentially hazardous fungicides and have been proved to be an efficient and a versatile competitor as they can be elicitors

or matrix component or even active constituent carriers. The antifungal property can be enhanced by effective amalgamation of antifungal agents with these biopolymers resulting in innocuous fungicides. Chitosan is one such polymer shown to be a potent fungicide that enables the plant to initiate defense mechanism against microbes, especially against fungal infections. Pretreatment of seeds with chitosan as a coating or even soaking has proved to be beneficial as it impedes the spore formation and mycellar growth while it improves seedling growth. Even nanoparticles based on chitosan exhibited antifungal properties, and edible coatings of chitosan are very potent alternatives for improving the shelf life of fruits like apples against microbes. Apart from this, coatings using alginates stimulate the development of different plant parts of both aerial and roots, consequently improving their resistance toward pathogens. Nanoparticle addition to these coatings can be used to improve their antifungal activity.

Cellulose and its derivatives have been found to be effective carriers and binders of active ingredients and control agents apart from being used as protective barriers and biodegradable plant membranes. Ether derivatives like carboxymethyl cellulose (CMC) are efficacious fungicidal seed coatings while hydroxypropyl methylcellulose (HPMC) are edible vegetable/fruit coating used in conjunction with preservatives. They constitute a class of promptly biodegraded polymers with finite shelf life, which requires a preservative that is integral to prolong their biochemical properties. Apart from this, such cellulose-derived biopolymers from various sources lack safety and efficacy profiling mainly due to polydispersity and structural heterogeneity arising from their sources and it is complicated to refine these raw materials as it is an expensive and incompetent process.

To summarize all three biopolymers, it was noted that even though cellulose is inexpensive and easily available its effective applications require better assessment procedures to cater to its disadvantages like high lipophilicity and safety concerns. Chitosan is an expensive alternative but is an effective antifungal agent, while alginate is very hydrophilic but has to be mixed with other microbes.

Degradable manure has advantages of not hindering the natural processes of successive plantations and fumigant mulches that effectively require films imparting low porosity, thereby decreasing application cycles of pesticides. Naturally occurring polymeric compositions that can enhance the pesticidal activity under ambient climatic conditions and pH are being designed, genetically tailored, and developed with improved shelf life, and a safer, broader spectrum of antimicrobial activity, which can be integrated into current pest management game plan.

Biopolymers for Biomedical Applications

Biopolymers are frequently employed in biomedical applications. They are employed in tissue engineering, pharmaceutical industry, medications, drug delivery, tissue engineering, pharmaceutical carriers, medical devices, and so on because of their features, including degradability and nontoxicity. Polypeptides are cheap and easy to come by, thus they are used in a variety of biomedical products. Biopolymers

can be used in a variety of biomedical applications when conventional polymers are ineffective. Biopolymers degrade in the human body and are devoid of any adverse effects, making them a feasible choice for medical use. Biopolymers have gained enormous recognition in recent years owing to their potential applications in biomedicine. Gelatin, for example, is a biopolymer commonly used in medicine for wound dressings, adhesives, and other purposes. In addition, simple porogen such as solvents or gases were used to make porous gelatin scaffolds and films. This procedure permits the scaffolds to contain medicine or nutrients that will be delivered to the wound to help it recover. Gums are used to encapsulate flavors, fragrances, phenolic compounds, antioxidants, and nutraceutical substances, among other things. The controlled drug release characteristic of gums enhances the targeted drug delivery mechanism. It is used to treat hypotension induced by surgical shock in the pharmaceutical business. It is also commonly employed in formulations as a tablet binder and emollient in cough drops and syrups (Yadav et al. 2015; Aggarwal et al. 2020).

In Drug Delivery

Synthetic polymers have long caught researchers' curiosity as encapsulating materials for a number of therapeutic chemicals. Despite the fact that they have better pharmacokinetics than small-molecule medications, the toxicity concern due to their buildup has caught enough attention (Schmaljohann 2006). The key challenge with the pharma industry's reorientation toward the custom utility of biopolymers is the choice of the appropriate compounds relying on the requirement and anticipated outcomes. It is critical that these biopolymer encapsulating agents safely deliver the active drug to the target effectively through oral or intravenous drug administration (George et al. 2019).

In the pharma manufacturing industry, alginate is arguably the most extensively utilized biopolymer, owing to its unique encapsulating and wound-patching properties. Alginate was initially utilized as an encapsulating agent in the treatment of diabetes to encapsulate pancreatic islet cells (Lim and Sun 1980), but alginate gels are too porous and cannot provide immunoprotection. Alginate gels must be treated with synthetic cationic polymers, namely, poly-L-lysine and poly-L-ornithine, which are utilized apart from glutaraldehyde, chitosan, PEG, and agarose.

The encapsulating ability of chitosan depends on crystalline nature, molecular weight, extent of deacetylation, and degree of ionization/free amino group. Chitosan prevents enzyme decomposition, controls discharge, and improves bioavailability when employed as a nanoparticle coating agent for nanoparticles utilized for brain disorders. It also affects tight junctions, which improves medication penetration across the blood–brain barrier (Yu et al. 2019). Furthermore, chitosan has high bioadhesive qualities and inhibits nasal drug release, and facilitates drug transport to the brain from the nasal cavity (Wang et al. 2008).

Agar derived from Rhodophyceae algae, is a long-chain biopolymer, constituting agarose and agaropectin (Mostafavi and Zaeim 2020). When agar functions as the

only encapsulating agent, the medication is released in two phases. The first and quicker phase results in the release of 10–20% of the medication depending on the extent of the agar content. The second phase is slower and lasts longer, and it gets slower as the disintegration progresses. The drug molecule remains on the surface and is present in the peripheral layer of the bead in the first phase, while in the second phase, the release is triggered by the solid core dissolution. The bigger the amount of agar in the beads when employed as an encapsulating agent, the matrix gets denser and it lowers drug molecule transport through the beads. Additionally, swift drug release occurs through beads with a lesser quantity of agar in formulation, resulting in large content of water (Rivadeneira et al. 2018).

As an encapsulating matrix, high crystallinity starch has been investigated. Starch can be altered to improve its mechanophysical properties, allowing it to be used more effectively in medicine delivery and other industries. Chemical, physical, enzymatic, and genetic mechanisms can all affect starch. Chemical processes are the most commonly employed since they are nondissolving and might potentially boost the functioning of the modified starch.

Carboxymethyl cellulose (CMC) remains one of the extensively utilized cellulose types owing to the properties being the most cost-effective and easy to spread. The presence of many hydroxyl groups in the structure of CMC creates hydrophilicity in the molecule that resists lipid oxidation and minimizes oxygen permeability. The metabolism of CMC in the digestive system limits its utility as probiotic encapsulation material. Biopolymer incorporation enhances the survivability of encapsulated anaerobic probiotics by 36% when combined with supplementary biopolymers like inulin, alginate, chitosan, gelatin, etc. (Zabihollahi et al. 2020).

Several constraints are still preventing the widespread application of biopolymers. First, most of the research has been done *in vitro*; consequently, additional biological testing in animals and humans is necessary to validate the therapeutic potential of biopolymers in diverse biomedical applications, particularly biocompatibility of drug delivery encapsulating materials (Zabihollahi et al. 2020). Studies are carried out by researchers to improve thermal resistance, mechanical properties, kinetics, and barrier properties to make materials that are equivalent to or superior to synthetic products. Some products, for example, have limited mechanical qualities, fast deterioration, and a high hydrophilic capacity, making them unsuitable for use in humid or unfavorable settings.

Extensive research in the area is required to make sustainable and permanent changes in the biomedical and pharma sector. Biopolymers have been shown to be successful as encapsulating materials in controlled drug delivery systems. Despite considerable improvement in biocompatibility, metabolic stability, and mechanothermal properties, the design of target selective carriers with diverse transport pathways and provide long-term release at the target site remains a hurdle (Udayakumar et al. 2021).

Despite present host restrictions, predominantly, the usage of polysaccharide-based polymers will continue to flourish in order to develop better drug interactions, biochemical and physiological transformations, and compatibility with target tissues. Although additional research is necessary to assess the complete potential of

polymers that are proved to be extremely useful for dispensing highly effective, biopotent, and safe small compounds.

In Tissue Engineering

Biocompatibility, biodegradability, non-immunogenic qualities, increased bodily fluid assimilation capacity, increased gel-forming ability, and antimicrobial and anticancer abilities are among the unique biophysical and biochemical properties of natural biopolymers. Such unique characteristics of biopolymers gave them an enviable stature in tissue engineering and other healthcare applications. Natural bone's outstanding mechanics are interconnected to its hierarchical pattern from the nano to the macro level, which can suitably arrange organic and inorganic components at the nanoscale level and periodically deposit collagenous substances during bone biomineralization. Hard and soft tissue engineering technology like cartilage restoration, regeneration of bone, and curing of wounds utilize a wide variety of biopolymers. Less mechanical, chemical, and structural qualities, as well as less biodegradation stability, are the key drawbacks of biopolymer tissue engineering. As a result, more research is required to change biopolymers in order to generate new flexible materials highly favored for tissue engineering applications. Currently, researchers are focused on constructing 3D scaffolds that may be used in tissue engineering applications to get superior results (Bedian et al. 2017; Babu et al. 2013; Chinga-Carrasco 2018).

Collagen is an important biopolymer that makes up 30% of the bulk of vertebrates. It is an adaptable biomaterial for neuronal repair and neural channel filling. Collagen hydrogels have proved to be a useful sciatic nerve repair material in rats. Collagen, when combined with proteins or other biopolymers, produces improved tissue regeneration results. The collagen-PGA tube has improved electrophysiological characteristics and is a promising material for nerve conduits in cats. The collagen framework enhances the rate of nerve repair by interlinking with laminin. Collagen remains the sole biopolymer that has been accepted for use in neuronal clinical examination (Cen et al. 2008).

Gelatin is an excellent denatured protein that is generated when animal collagen is hydrolyzed in an alkaline or acidic environment. Gelatin is a cheap, readily available sustainable biomaterial. Denatured gelatin shows lower antigenic properties than collagen, while the proliferation and variety of cell adhesion lead to the enhancement of biological qualities of the implanted polymeric device due to the changing chemical structure. The ideal biochemical, kinetic, and mechanical properties woven along with other synthetic and natural polymers are an integral part of functional frameworks. Gelatin nanoparticles are a sought-after biomaterial for neural tissue regeneration mainly due to their superior biocompatible nature. The acetate/PLA molecular framework support coated with gelatin nanoparticles exhibits higher cell viability than the uncoated materials. Development, cell adhesion, regeneration, and propagation of neurons are stimulation by gelatin–chitosan–PEDOT hybrid scaffolds. Regeneration, curing, and repair of damaged or infected tissues and

organs require a versatile biopolymeric material like collagen (Nikkhah et al. 2016; Salerno et al. 2018).

Flexibility, long-term stability, self-assembly, and interesting biological activity are the hallmarks of elastin. Apart from providing elasticity to tissues, it is also present in blood vessels, lungs, skin, and ligaments and has been found to be an important material for neurodegenerative diseases that can mend precise fragments of CNS (Ozsvar et al. 2015).

Hyaluronic acid (HA) consists of glycosaminoglycan derived from extracellular tissues in the human body, which plays a major role in lubricating and can be chemically manipulated apart from being biodegradable and biocompatible. The receptors present on the periphery of stem cells interact with HA and help in controlling various cellular activities like survival, motility, proliferation, intracellular signaling, as well as transduction and differentiation. Hence, HA-derived biopolymers have been extensively utilized in biological testing as injectable hydrogels and bioscaffolds, with promising results for tissue regeneration. HA hydrogels have the ability to boost the survival rate and neural precursor cell proliferation, which has significant implications for peripheral nerve regeneration treatments and CNS repair techniques owing to its versatile mechanical properties that can regulate neuronal networks and thus opening up a novel avenue for treating neurodegenerative illnesses (Makvandi et al. 2020; Hemshekhar et al. 2016).

Keratin is a polypeptide protein made up of a variety of nonpolar and polar amino acids linked together by both intra- and intermolecular molecular bonding. Keratin plays an integral role in facilitating cell proliferation and adhesion and is the primary biomaterial to be employed in neural tissue engineering that permits Schwann cells to infiltrate and proliferate more easily. Human hair-derived keratin hydrogels are efficient in treating fatal liver injury (Imam et al. 1999; Balasubramaniam and Chinnan 1997). Keratin's main disadvantages as a tissue engineering material are its low mechanical strength and complications encountered in handling high sulfur and protein content. The dissolution and abstraction of this biopolymer remain a huge challenge so far when compared to others in the same category (Lin et al. 2019; Rajabi et al. 2020; Shavandi et al. 2017).

Lower mechanical, chemical, and structural qualities, as well as less biodegradation stability, are the key drawbacks of biopolymer tissue engineering. As a result, tissue engineering research is essential to incorporate biopolymers to generate novel flexible materials better suited for tissue engineering applications. The current focus of researchers lies in constructing 3D scaffolds that may be used in tissue engineering applications to get superior results.

In Medical Implants

These biopolymeric materials are ideal candidates for implantable materials because of their biocompatibility, biodegradability, and lack of cytotoxicity. Implantable devices are designed to look like a bodily part and are used to substitute an injured organ or structure in order to maintain ordinary bodily functions. Medical implants

of important organs like the eye, heart, hips, etc., utilize ceramics, metals, and synthetic polymeric materials that are found to have several drawbacks, including immune system rejection arising from the biodegradability of synthetic polymers (Bhatt and Jaffe 2015). The metabolic processes like hydrolysis produce carbon dioxide, which in turn lowers the cellular pH, resulting in cellular necrosis (Liu et al. 2006). Biopolymers introduced as medical implants offer biodegradability and play a significant role in this arena.

PLA remains one of the potent biodegradable polymers possessing cost-effectiveness and mechanical properties similar to polypropylene (Mukherjee and Kao 2011). Furthermore, PLA is biocompatible, which is an added advantage when being used as implantable devices that will degrade, leaving no foreign body, which will circumvent the requirement for a second surgery to remove the device after its lifetime. PLA-incorporated biodegradable stents have shown great potential in preclinical and clinical studies (Hietala 2004). PLA-PGA copolymer-based devices are more beneficial than devices bearing respective copolymers and are employed as orthopedic implants to treat bone deformities and fractures. Framework building in the body to help cartilage construction also utilizes biopolymers (Rebelo et al. 2016).

Meinel and Kaplan (2012) utilized silk fibroin for scaffolding purposes in the musculoskeletal system with excellent mechanical competencies, exceptional stability, and precise delivery of protein and small-molecule drug molecules. Silk fibroin-derivatized scaffolds and their protein–drug bioconjugates help in fine-tuning the drug delivery through microneedles in mimicking biological processes. Forthcoming research results in the arena of biopolymers targeting skeletomuscular diseases are predicted to be prompted by potential developments in drug delivery/biomaterial interaction.

Chitin or poly-*N*-acetyl-*D*-glucosamine polycationic derivatives existing in microbial cell walls and envelope of crustaceans are generically known as chitosan. Implants derived from chitosan include stents, bone, cartilage, skin, liver, etc. The researchers have experimentally proved the ability of a combination of chitosan and heparin to rapidly increase the recuperation process in vivo in pigs post-stent replacement (Meng et al. 2009). Chitosan-derived films have also shown excellent mechanical stability when mixed with an alginate framework and have been reported to create an osteoconductive matrix (Chen et al. 2009; Li et al. 2005).

These biopolymers have intrinsic features, such as biodegradability and biocompatibility, offer significant benefits, and expand their potential usage in implantable medical applications. These advantageous features place them superior to their synthetic analogs, and it has to be noted that a combination of these biopolymers with synthetic counterparts has the potential to revolutionize the medical field.

In Wound-Healing Materials

The ability of biopolymers to absorb significant amounts of water while dry and donate water when hydrated is one of their most valuable characteristics. Medications impregnated with formulated biopolymers are both target-specific and controlled drug release and have been widely operational in tissue delivery and

rebuilding. Wound-healing dressings are classified according to their components to hydrocolloid, alginate, or hydrogel dressings in the reported literature. Alginate, being a hydrocolloid with the ability to create hydrogels, may plausibly fit into all three groups from a material standpoint. This ambiguity should not be misunderstood as wound-dressing classifications refer to the precise type of dressing used to treat certain types of wounds. Interactions of these biopolymers with the wound tissue, and stability and molecular mass of these biological polymers are not completely understood yet (Nyström 2016).

Collagen is recurrently employed as a hydrogel that aids wound healing by transporting anesthetics, growth factors, medications, etc. Integration is frequently successful as it is a substrate that thwarts rejection due to the fact that it is naturally occurring and cell attachment prefers it. Collagen creates hydrogels resembling skin and is very helpful in wound healing by transporting the mesenchymal stem cells to the wound (Ruszczak 2003).

The hemostatic character of gelatin coupled with other biopolymers is utilized as hydrocolloid wound dressings, and DuoDerm[®]/Granuflex[®] (ConvaTec) and Gelfoam are some of the trade names under which it is marketed. Despite having unique features that make it ideal for producing wound-healing dressings, gellan gum remains an underutilized polysaccharide. Gellan consists of a linear tetrasaccharide repeating unit that comprises two glucose units, rhamnose, and glucuronic acid, and is generated during aerobic fermentation of *Sphingomonas paucimobilis* (Smith et al. 2007; Sworn et al. 1995).

Another commonly exploited polysaccharide in wound healing is alginates. The calcium, sodium, magnesium, and alginic acid salts were isolated from seaweed (Phaeophyceae). The major purpose of employing an alginate dressing is to control wound exudate. When wound exudate comes into touch with alginate, the sodium ions in the exudate ion exchange with the calcium in the alginate, allowing the alginate to dissolve. This is because calcium is less tightly coupled to mannuronate than it is to guluronate, and thus solubilizes more rapidly. The development of drug-impregnated alginate wound dressings and composites with other biopolymers, resulting in multifunctional dressings, has been a focus of further advancements in alginate wound dressings (Jones 1999).

Biopolymers possess multifunctional activity and adaptability to various physical states, enabling them to be used in a broad range of types of wounds, from intertwined fibers that can help dry wounds by swelling when they interact with water-rich hydrogels. Physiologically receptive smart biopolymers are developed owing to their biocompatibility and drug delivery capacity when it comes in contact with varied physiological conditions. There exists a huge demand for a biomimetic and sustainable medicine approach to wound healing, in particular, it opens the door to the development of complex biopolymer products that combine medicines, wound-healing proteins, and cells. Furthermore, personalized medicine is the futuristic view amalgamated with the innovations in genomics, proteomics, and stem cell technologies. Potentially it transforms wound-healing treatments, and abundant advantages offered by biopolymers will almost certainly pave a way for the development and delivery of these treatments in the future (Boateng et al. 2008).

Outlook and Perspectives

Worldwide production of synthetic polymer crosses over 140 million tons per year. Synthetic polymers being very stable remain in biosphere for a very long time, creating various long-lasting environment pollution depending on their hydrophilic and hydrophobic nature. Water-soluble man-made polymers in wastewater and plastics have proven to dangerously interfere with food chain and result in biomagnification and are treated to be the major cause of extinction of certain species. Toxicity of these persistent organic pollutants is a grave issue that has been recognized by researchers across the globe. The natural polymeric materials and their derivatives have gained more priority owing to their biodegradable and nontoxic nature while not compromising on the effectiveness of their utility if not bettering the properties of synthetic polymers. Hence, current research is mainly centered on creating novel biodegradable polymers with enhanced applications. Most of the biopolymers are produced or formed during the life cycle of organisms involved in their production. Renewable materials as opposed to the nonrenewables have been in high demand due to various reasons discussed earlier in this chapter.

Plastics mainly utilized for packaging like styrofoam cause immense terrestrial and water pollution. This pollution is mainly linked to health problems in humans and animals, resulting in major life-threatening diseases and affecting the marine and terrestrial quality of life.

Scientists and technology experts are conscientiously creating innovative processes and novel biopolymers that can address the aforementioned disadvantages without compromising the quality. Such polymers have found their applications in different fields like agriculture, cosmetics, nutrition, healthcare and packaging, etc. Development of various biomaterials requires stringent research methodologies and analytical testing to evaluate the safety, effectiveness, toxicity, and environmental degradability. Once they cross this testing, they can replace synthetic polymers as a sustainable alternative and can be integrated into society for a healthier, safer lifestyle.

Researchers focus mainly on biodegradable characteristics while they develop novel biomaterials or biodegradable polymers apart from their antimicrobial potential. An array of biomaterials are being utilized to produce sustainable alternatives to polymers making use of various processes. For instance, fermentation of sustainable crops like corn and sugar beets yields biopolyesters like polylactic acid or PLA arising from the polymerization of lactic acid resulting from the fermentation process. Polymerization of ϵ -caprolactone is the raw material for another such polyester biomaterial PCL, which is also known as polycaprolactone with low viscosity, low melting point, and better thermal modulation. Stress resistance and adhesion properties are modulated by mixing PCL with other biopolymers. Polyhydroxy butyrate (PHB) and polyhydroxyalkanoates (PHA) are other examples of biopolymers frequently exploited for packaging of food.

A variety of techniques and materials are utilized to design and produce an assorted array of biodegradable and sustainable polymers unlike conventional

polymers that are mainly produced from volatile, toxic, and nondegradable petroleum resources. Newer biodegradable polymers ought to be fashioned with sustainable properties embedded in them so as to be used for biomedical operations, especially drug delivery vehicles/systems, tissue engineering porous 3D scaffolds, medical implants, and temporary prosthetics that cater to a variety of medicinal purposes like regenerative medicine, stimuli dependent hydrogels, etc.

Another arena wherein these biomaterials have found utility is their ability to be used as bioemulsifiers in food industry, which is estimated to be a 17.53 billion market in the next 5 years. Liposan is a well-known example derived from *Candida lipolytica*, which is utilized to improve the stability of vegetable oil-water emulsions.

Clearly, not all biopolymers are bona fide biopolymers. For engineering applications, it is typically a mix of oil-derived polymer and genuine biomass-derived polymer. The market is increasing, but at a rate that is about in pace with the broader plastics industry, implying that oil-derived products are not being displaced in any significant way. Biopolymer demand accounts for less than 1% of total global polymer demand. If biopolymers and those who make them are to have an impact on the environment, they must improve. Biopolymers are now being developed for usage in a plethora of industries, extending from simple uses to high-use applications such as medicine. Biopolymers are rapidly expanding in popularity due to their biodegradability, and these materials are being developed for a variety of uses. Biopolymers are being used in a varied array of applications, as evidenced by their market growth of over 23% per year since 2010. The market growth pattern is predicted to linger for a while, according to GBI Research. With the growing need for green energy and ecologically friendly materials, biopolymer manufacturing should increase, and the use of these materials should become more widespread to replace non-degradable polymers. Biodegradable biopolymers are expected to account for a bigger proportion of biopolymer manufacturing in the coming years. Manufacture is expected to rise almost twofold during the last 5 years (from 2.27 million tons in 2017 to 4.31 million tons in 2022), indicating the slow pace at which it travels in the journey of replacing petroleum products. Public demand and government policies create an impact on such a drive.

In many circumstances, the cost of bio-based products outweighs the cost of petrochemical ones. Biopolymers, on the other hand, have several drawbacks, including their hydrophilicity, which results in inferior mechanical characteristics in humid settings. As a result, we must find a solution to these issues. As a result, additional research is required to improve expertise in biopolymer engineering to develop new high biopolymer materials for specific applications and increased use. The nanofiller additions to these biopolymers, for example, can enhance the mechanical and barrier qualities of the biopolymer under study. This enhancement will aid in the expansion of numerous applications of biopolymers. The impending challenge of biopolymers is to completely substitute the use of petrochemical-based polymers in all industries as we come to the fag end of the “plastic era.”

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Biopolymer-Based Nanocomposites for the Treatment of Organic Pollutants from the Synthetic Wastewater

24

Application

Ruksana Sirach and Pragnesh Dave

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Abstract

Recovery of wastewater has become a major concern owing to a decline in freshwater availability. Among the various methods available for the treatment of wastewater, the adsorption route is a simple, cost-effective, and efficient way to eliminate hazardous water contaminants from wastewater, increasing the availability of potable water. The present chapter introduces the readers to biopolymers-based nanocomposites as an adsorbent to remove various hazardous contaminants from the aqueous stream like dyes, phenols, pharmaceutical drugs, and pesticides. The chapter will attempt to convey to its readers the need for wastewater treatment and the use of biopolymers-based nanocomposites as sustainable and eco-friendly adsorbents. Throughout the chapter, chitosan, cellulose, starch, sodium alginate, and β -cyclodextrin-based nanocomposites for the removal of various organic contaminants will be introduced. The various adsorbate-adsorbent interactions responsible for the adsorption and effect of inorganic ions on the adsorption efficiency of biopolymer nanocomposites are also provided.

R. Sirach · P. Dave (✉)

Department of Chemistry, Sardar Patel University, Gujarat, India

Keywords

Nanocomposites · Biopolymers · Wastewater · Adsorption · Removal

Introduction

Population and financial expansion have accelerated the pressure on water supplies. Consequently, 36% population of the global total currently resides in water-stressed areas. Urban development has caused a variety of water-related difficulties, notably in middle- and low-income nations, such as poor water quality and insufficient water supply facilities, especially in developing peri-urban and unorganized communities. Nations are launching major wastewater collection and treatment schemes to ameliorate the countries' wastewater issues (Elkady et al. 2020; Rodriguez et al. 2020). The area of the water supply has substantial investment requirements. As urbanization increases, there seems to be a chance to make expenditures most effectively and sustainably feasible. Prospective urban development will necessitate measures that reduce resource use while emphasizing resource recovery, in accordance with sustainability criteria. Wastewater is and should be seen as a precious commodity for extracting nutrients and energy, and a supplementary supply of water (Rodriguez et al. 2020). Wastewater may be processed to various quality levels to meet the needs of various industries, notably farming. It may be treated in an eco-friendly way and sometimes even repurposed as drinkable water. Treatment of wastewater frees up precious freshwater for alternative applications or conservation. Furthermore, treating wastewater effluent can be helpful for farming and power production, making wastewater treatment plants more ecologically and economically sound (Rodriguez et al. 2020). Polluted water is caused as a result of the presence of organic and inorganic contaminants that render the freshwater unfit for drinking and leave it with no further use (Thirunavukkarasu et al. 2020). To diagnose the polluted water, conventional chemical, biological, and physical methods were used. However, these approaches are most often regarded as being less effective in meeting water quality objectives. More specifically, these systems include drawbacks such as oxygen diffusive resistances, the building of stable lagoons, the discharge of waste containing harmful constituents, partial pollutants elimination, and lots of energy, cost, and time requirements for operation. The contaminated water has been diagnosed using the following methods, but each method has certain limitations (Table 1) (Li et al. 2020; Thirunavukkarasu et al. 2020). Wastewater contaminants can be into two classifications on the basis of their hazardous effect: (i) the black list: which includes harmful prioritized compounds that are chronic, extremely poisonous, or cause bioaccumulation and (ii) the grey list: which collects prioritized compounds that pose a severe environmental danger. These can be regrouped according to specific substances such as metals, phenols, nitro-compounds, or on the basis of the industrial sector such as metallurgy, food, pharmaceutical, dye, and agro-industries (Crini and Lichtfouse 2019; Li et al. 2020). Wastewater released from

Table 1 Methods for the treatment of the contaminated water

Method	Examples	Treatment
Primary treatment	Flootation, screening, centrifugal separation, filtration, coagulation, and sedimentation/gravity separation	Water is treated at the primary level, i.e., for the pretreatment of water before applying other purification
Secondary treatment	Biological methods such as aerobic and anaerobic treatment	Microbes that can transform contaminants into smaller and harmless compounds
Tertiary treatment	Ion exchange, electrolysis, oxidation, adsorption, reverse osmosis, electrodialysis, and precipitation	Includes chemical methods that have the potential to improve the quality of water to drinking water quality

the industrial sector can contain various contaminants according to their origin; the specific contaminants may need a different treatment.

Metal and organic contamination are among the most common environmental issues. They are nonbiodegradable and, in particular, have an elevated aqueous solubility, which facilitates their environmental mobilization by interacting with biotic and abiotic factors, such as adsorption onto organic colloids or buildup by life forms, posing a hazard to public health and ecological systems (Li et al. 2020; Lofrano et al. 2016). Hundreds organic contaminants, ranging from simplest to more intricately organized molecules, have been recognized as carcinogenic. Contaminations must be removed because they are hazardous to people and marine organisms when exposed to quantities over a certain threshold. Furthermore, the occurrence of these contaminants is unavoidable since these constituents are emitted by everyday items such as plastics, soaps, detergents, dyes, petroleum, and insecticides (Rout and Jena 2022; Thirunavukkarasu et al. 2020). In the presence of large quantities of organic contaminants, the dissolved oxygen in the water may be consumed at a faster pace than it could be replaced during the degradation/conversion of organic contaminants, resulting in oxygen deprivation and severe consequences for the organisms. Organically polluted wastewater comprises huge amounts of suspended matter, which restricts the amount of sunlight accessible to photosynthetic organisms making it undesirable for several creatures (Rashed 2013). Among several methods available for wastewater treatment such as ion exchange, reverse osmosis, photocatalytic degradation, adsorption, etc., adsorption is the simplest and most cost-effective. Ion exchange and reverse osmosis are often more appealing procedures since pollutant levels possibly be retrieved altogether with elimination from industrial effluent. But these methods require high investment and operational costs, and hence are not financially sustainable. Adsorption has the ability to be one of the most productive ways for the treatment and removal of organic pollutants in treating wastewater. Adsorption has an advantage over other technologies because of its simple design and cheap investment, making it economically sustainable (Feng et al. 2017; Mashile et al. 2020; Rashed 2013; Rout and Jena 2022). Biopolymers are lipopolysaccharides, glycolipids, proteins, and polysaccharides derived from biological sources such as microorganisms, plants, and animals. Biopolymers have additional benefits such as nontoxic nature, hydrophilicity, biocompatibility,

renewable nature, biodegradability, abundant functional groups, and availability (Heydari et al. 2018; Rout and Jena 2022; Sheen Mers et al. 2022). Furthermore, biopolymers are versatile and could be intercalated into complex configurations to enhance their characteristics or can be used as a matrix in which various other nanoscale materials can be reinforced to improve the characteristics of the existing biopolymers. They also have abundant reactive functional groups such as “–OH,” “–COOH,” “–SO₃H,” and “–NH₂” along with electron-withdrawing, hydrophilic, and hydrophobic sites, leading to varied porous polymeric networks. By adjusting the pK_a of the polymeric matrix, one can design the surface of a biopolymer with desired functional groups to eliminate particular contaminants. As a result, the biopolymer and its variants could be used to eliminate organic molecules (dyes, oils, phenols, etc.) and inorganic species (heavy metals), and other contaminants from wastewater effluents (Sheen Mers et al. 2022). Biopolymer-based nanocomposites have several inherent benefits against artificial polymer-based compounds, like being sustainable, biocompatible, biodegradable, physically resilient, and multifunctional (Sheen Mers et al. 2022; Xiong et al. 2018). Even though every one of these primary biopolymers has flexible and quasi-primary structures, secondary and tertiary organization of the biopolymers, as induced by complementary weak intramolecular and intermolecular bond formation, stowing, or knotting, is expected to present improved physical and functional efficiency for demanding applications (Xiong et al. 2018). Bio-based nanocomposites, because they symbiotically integrate the strength and functionality of individual components to yield new nanosystems with augmented utilities such as environmental sustainability, low toxicity, environmentally friendly, and processing biocompatibility, advanced bio-derived nanocomposites with advanced functionalities that open a promising pathway forward into producing the attributes essential to accommodate the changing demands of current technological advancements. These nanostructures’ improved biocompatibility makes these materials great prospects for a wide range of demanding human-technology advancements (Sheen Mers et al. 2022; Xiong et al. 2018). Thrown bio-nanocomposites break down rapidly to their lower basic components. As a consequence, the regenerative, biodegradable, and sustainable processing properties of biopolymeric materials have the potential to considerably cut CO₂ emissions and toxic waste. To obtain or improve on these environmental advantages, distinct functional nanocomponents with significant and complementary interactions with biopolymers should be included in the matrix material, to produce superior mechanical improvements or additional functions (Xiong et al. 2018).

Adsorption

Adsorption is a process in which pollutants from a liquid or gaseous carrier get deposited/concentrated on the surface of a solid known as an adsorbent, the constituents getting concentrated/deposited/adsorbed on the layer of the adsorbent are known as adsorbate. The phenomenon is known as adsorption (Nakhjiri et al. 2021). The adsorbate can form a single layer or it can form a multilayer on the adsorbents’ surface.

The adsorption performance is affected by many factors some of which are given below in brief.

Temperature: The removal efficiency of adsorbent may increase or decrease with varying temperatures depending upon the nature of the adsorption process. If the adsorption process is endothermic, then the removal of adsorbate gains heat from its surroundings, and an increase in the temperature will facilitate the adsorption of pollutants on the adsorbent. On the other hand, the contradiction is obtained if the adsorption process is exothermic (releases heat into the surroundings). The exothermic nature of the adsorption process leads to decreased removal efficiency of pollutants by the adsorbent. The majority of (nearly all) adsorption processes are exothermic as the adsorption of pollutants on the adsorbent surface decreases the energy of the adsorbent and hence excess energy is released in the form of heat.

pH: pH of the solution directly influences the adsorption performance depending upon the nature of the adsorbent and adsorbate. The variation in the pH of the solution can either protonate or deprotonate the surface of the adsorbent resulting in the generation of a cationic or anionic surface, which can improve or diminish the adsorption of pollutants depending upon the anionic or cationic nature of the pollutants.

Adsorbent amount: Increasing doses of the adsorbent leads to enhanced adsorption of pollutants. However, in selected cases, if the adsorbent is highly agglomerated, the adsorption may decrease with increasing adsorbent dose as a result of the decreased available active surface area and pores of the adsorbent.

Adsorbate (pollutant) amount: The dose of adsorbate may increase or decrease the removal of pollutants from the wastewater depending upon the saturation of active sites of adsorbent. After the saturation of active sites, increasing adsorbate content will decrease the % removal efficiency.

Contact time: The adsorption of the pollutants on the surface of the adsorbent increases until the equilibrium is established. The pollutants may get released from the surface of the adsorbent into the solution if the adsorbent is kept for a longer time in the solution.

Nature of adsorbate and adsorbent: The functionality, surface area, pore size, and surface charge of adsorbent on adsorbent influence the overall adsorption performance. The structural features (functionality and charge) can also influence the adsorption efficiency.

Dyes

Paper, textiles, leather, beauty products, food, and plastics are few of the industry sectors that use dyes (colored organic compounds) to color their products. Dye is an organic compound that may get attached to the surface of a material such as fabric or paper and impart color to that material. About 10–15% of all dyestuffs are lost directly to wastewater during dyeing procedures. The wastewater discharge containing dyes creates an eco-toxic threat and poses a risk of bioaccumulation

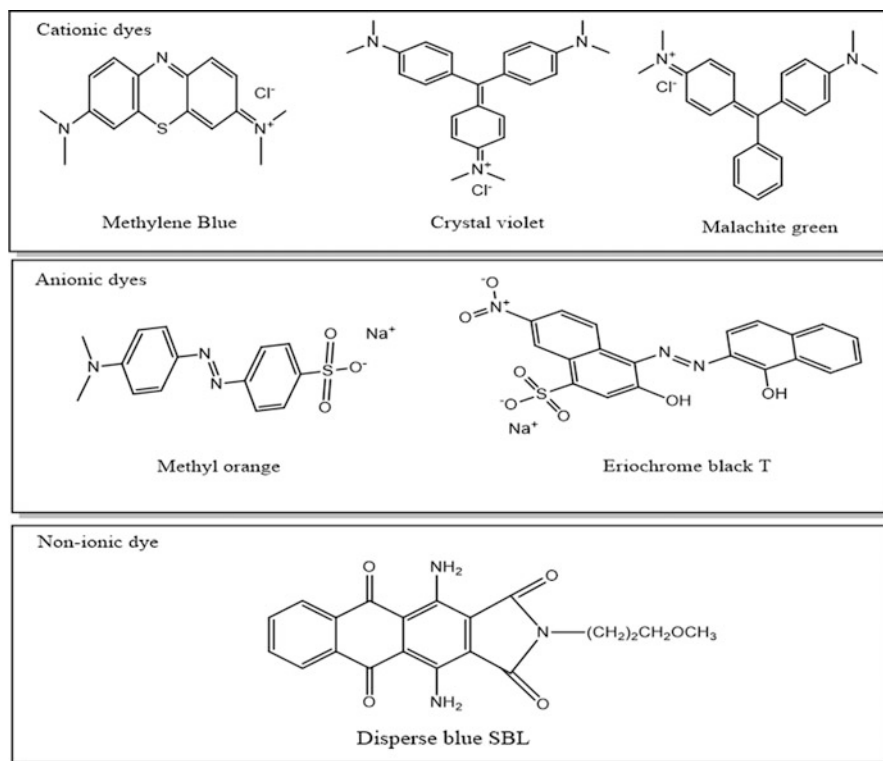


Fig. 1 Structure of a few cationic, anionic, and nonionic dyes

(Yeamin et al. 2021). The dyes can be classified into three major groups depending on their ionic charge (Fig. 1):

- (i) Anionic dyes: Consist of an organic moiety having a negative charge, hence produces organic negative ions when dissolved in water. Direct, reactive, and acidic dyes are anionic dyes. Because of anionic dyes' low degree of fixation owing to reactive group hydrolysis in water, releasing reactive dyes into the ecosystem causes severe hazardous effects (Kumar et al. 2019).

Examples: Methyl orange and Eriochrome Black T

- (ii) Cationic dyes: This group of dyes consists of a positive organic moiety that has a number of chemical structures based on their substituted aromatic groups. Wool, silk, nylon, and acrylic fabrics are majorly dyed using cationic dyes. Cationic dyes produce colored cation in water, and because of their ionic nature they are water soluble, and hence can badly influence the quality of water. The cationic dyes adversely affect health causing genetic disorders, cancer and dermatitis, and skin irritation. The Triarylmethane, azo, and anthraquinone dyes are cationic in nature (Kumar et al. 2019).

Examples: Methylene blue, crystal violet, and malachite green

- (iii) Nonionic dyes: The dyes included in this class does not possess charged ions in their structure.

Examples: Disperse Blue SBL

In humans, greater levels of these dyes cause cardiac problems, shock, vomiting, quadriplegia, tissue necrosis, and jaundice. The presence of pigments in the aquatic environment leads to the coloring of water and oxygen depletion, causing harm to animals as well as plants (Kumar et al. 2019). To overcome the environmental impact of dyes present in the wastewater, it is essential to remove the dyes from the wastewater. Biopolymer-based nanocomposites are extensively studied for the removal of dyes from wastewater.

Among the most substantially used biopolymers for synthesizing biopolymers-based nanocomposites included polysaccharides like starch, cellulose, chitosan, etc. Chitins' production originating from clam exo-skeletons is estimated to be just about equal to that of cellulose. Chitosan is produced by the deacetylation of chitin and is composed of β -(1-4) glycosidic bonds of D-glucosamine and N-acetyl-D-glucosamine. Cellulose is the most abundant polysaccharide followed by chitosan, which makes the nanocomposites based on cellulose and chitosan cheaper. Cellulose originating from vegetable sources such as grasses, stalks, woody plants, and reeds is made up of repetitive β -(1-4) D-glucose linked together by glycosidic linkage (Kanmani et al. 2017; Mashile et al. 2020; Sheen Mers et al. 2022). The low surface area, soft structure, and low mechanical strength limit the direct application of chitosan as an adsorbent. The hydroxyl and amine as well as hydroxyl functionality on the cellulose and chitosan, respectively, make their functional modifications possible for the adsorption of organic pollutants. However, when chitosan is dissolved in an acid medium, it agglomerates and gels, making it problematic to distribute/disperse and preventing -OH and -NH₂ groups to form chelated structures with cationic species (Fathy et al. 2020; Kanmani et al. 2017; Li et al. 2020). The three-dimensional (3D) porous structure enables easy and faster diffusion of adsorbate molecules on the adsorbent. Nanoclays are among the suitable adsorption materials owing to features such as swelling, layered microporous structure, large specific surface area, nanoscale size, capacity for cation exchange, and cheaper price compared to traditional adsorbents, i.e., activated carbon. Nanoclays are also appropriate for large-scale applications because of their high safety and stability. However, owing to the clays' solubility in water, inorganic clays are ineffective for the adsorption of lipophilic contaminants from water. Nevertheless, by intercalating alkyl linear chains or chemical bonding into the diffusion layer, the nature of the diffusion layer may be changed from hydrophilic to lipophilic, providing clay minerals with a diverse range of novel and fascinating adsorption characteristics. Because clays are biocompatible and biopolymers are biodegradable, their combination is an effective technique for the manufacture of bio-nanocomposites that may be utilized for pollutants removal (Orta et al. 2020). The synergy and control of interaction between biopolymers and clay in nanocomposite may help in producing novel smart composite material for the environmental applications. Hydrogel nanocomposites are 3D polymer frameworks that may be used independently in a wide

spectrum of uses as they retain a huge amount of water because of physical or chemical cross-linking among distinct polymer network, and they may soak huge amounts of water yet retain water insolubility (Bardajee et al. 2016). Salep, a hydrophilic, environment-friendly polysaccharide obtained from terrestrial orchids, possesses the ability to alter its swelling properties. Salep grafted by a polyacrylic acid hydrogel network reinforced with silver (Ag) nanoparticles follows a multilayered adsorption process for the adsorption of rhodamine cationic dye, indicating the potential of a physical adsorption mechanism. At 30 °C temperature, the highest adsorption capacity of the nanocomposite adsorbent was 92.59 mg g⁻¹. The presence of nanoscale Ag in the hydrogel establishes a coordination link with rhodamine's electron-rich groups, e.g., amine $\rightarrow\text{C-NH}_2$, and functional groups like C=NH_2^+ , O, and C-NH_2 interact with the hydroxyl (-OH) and carboxylic (-COOH) groups of the polymer matrix in the nanocomposite via hydrogen and polar interactions, promoting adsorption of dye. The presence of silver nanoparticles in the polymer network also enhances the porosity and free regions, resulting in significant swelling capability and dye adsorption (Bardajee et al. 2016). One of the most studied dyes for adsorption is methylene blue, excess amounts of methylene blue in an aqueous medium can cause skin and cardiovascular issues. On a long-term exposure, it is considered carcinogenic and is not easily degradable (Mahmoud et al. 2020). Several studies have focused to eliminate methylene blue from wastewater effluent using adsorption treatment utilizing biopolymer nanocomposites. Polyacrylic acid cross-linked chitosan reinforced with titania (TiO₂) shows increased swelling and adsorption capacity for methylene blue with increasing % TiO₂ content. The elevation in % TiO₂ concentration in the polymer matrix may decrease the density of cross-links in the resulting composite and increase the porosity of the nanocomposite material, and as a result more water may be absorbed due to free volume/space accessible in the polymeric network (Mahmoud et al. 2020). Montmorillonite is among the most widely utilized clays to reinforce the polymeric matrices. It is an aluminum phyllosilicate composed of aluminum oxide and silica layers arranged in an octahedral and tetrahedral geometry, respectively. The replacement of Al³⁺ ion present in octahedral sheet with isoelectronic Mg²⁺ results in the net negatively charged surface. This negatively charged surface is stabilized by exchangeable positively charged hydrated cations (Ca⁺² and Na⁺). Therefore, the clay has characteristics like swelling capability, cation exchange capacity can adsorb varying pollutants in its interlayer spacing, or electrostatic interaction with cationic charged polymer surfaces. The exfoliation of the clay sheets can enhance the specific surface area of the resulting nanocomposite. These properties enable clay-polymer-based nanocomposites a low-cost, more efficient, eco-friendly alternative to the conventional adsorbents. The varying amount of chitosan (reinforcing materials) from 50–300% of the cation exchange capacity of clay leads to the increased adsorption capacity of nanocomposite from 98–282.01 mg g⁻¹ for reactive violet 5 (an anionic dye) (da Silva et al. 2021). Natural clay modified with starch and MnFe₂O₄ nanofiller enhances the surface roughness and the active surface area of natural clay five times compare to pure clay without any modifications. The porosity is also an important factor in adsorption. The pore size of the resulting clay/starch/

MnFe₂O₄ nanocomposite was improved from 3.80–4.06 nm. The increased specific surface area and pore size of the nanocomposite allow dye molecules to penetrate more easily within the layers, resulting in 15% better adsorption performance of the clay/starch/MnFe₂O₄ nanocomposite than the clay for the adsorption of sunset yellow (anionic dye) and Nile blue (cationic dye) (Esvandi et al. 2020). Similarly, xanthan gum/Methionine-functionalized bentonite nanocomposite shows enhanced surface area than the bentonite clay. The composite exhibits a maximum capacity of 530.549 mg g⁻¹ for Congo red, a carcinogenic anionic dye (Ahmad and Mirza 2017). Sufficient interaction between biopolymer and nanofiller is of great importance for the performance of resulting nanocomposite. The polarity, large number of active functional groups, geometry, and aspect ratio are among the factors governing the interaction between matrices and fillers (Rouf and Kokini 2016). The adsorption capacity of nickel oxide (NiO)-reinforced chitin nanocomposites adsorb malachite green with a maximum adsorption capacity of 370.37 mg g⁻¹, which was not remarkable but the biodegradable nature of chitin in nanocomposite can provide a green alternative (Raval et al. 2016). TiO₂ nano-reinforced chitosan cross-linked with epichlorohydrin can adsorb reactive red 120 dye, an anionic azo dye (maximum adsorption capacity = 210 mg g⁻¹). The adsorption of reactive red azo dye on the nanocomposite could be the result of hydrogen bonding, electrostatic interactions, and/or π - π stacking (Jawad et al. 2020). The incorporation of magnetic nature in the biopolymer nanocomposite allows the easy separation of composite material after the adsorption treatment.

Graphene oxide (GO) is one of the most widely studied carbon nano-material-based adsorbents owing to its great chemical stability, large surface-to-volume ratio, surface functionality, and environmental friendliness.^[1] GO in an aqueous medium tends to agglomerate restricting their application as an adsorbent. Whereas GO has significant adsorption capabilities for a wide range of pollutants, its applicability is restricted as a result of the centrifugation force required after adsorption and GO's possible hazardous consequences. This limitation of GO can be avoided by reinforcing GO in matrices. Biopolymers might be employed to alleviate the constraints of GO in water pollutant removal (Feng et al. 2017; Heydari et al. 2018; Kanmani et al. 2017; Zaman et al. 2020). Natural biopolymers' large number of functional groups and stable macrostructure allows them to trap a variety of organic pollutants. Combining properties of biopolymers and GO in nanocomposite can provide an eco-friendly adsorbent with better performance, i.e., the presence of several -OH groups on cellulose (four portions) surface allows better dispersion of GO (one portion) in cellulose. The negative charge on the cellulose because of -OH functionality increases the interaction of the cationic dye with nanocomposite and the large surface area of GO can enhance the available active surface of nanocomposites resulting in better adsorption than the individual GO and cellulose material. The maximum adsorption of GO/cellulose nanocomposite for methylene blue adsorption was 751.88 mg g⁻¹. The GO/cellulose nanocomposite can be reused for up to seven cycles without major loss in the removal adsorption proficiency, after which a decline of up to 82.76% was observed on the tenth cycle. Loss of a small adsorbent amount during the regeneration could also appoint to some loss in the

removal efficiency during recycling (Zaman et al. 2020). In another study, the impregnation of aniline on the resulting chitosan/montmorillonite was achieved followed by polymerization to yield chitosan–montmorillonite/polyaniline nanocomposite for the removal of methylene blue. The maximum adsorption capacity was 111 mg g^{-1} , not great compared to other studies but yet higher than the individual components of the nanocomposite (chitosan = 28.1 mg g^{-1} ; polyaniline = 9.21 mg g^{-1} ; and montmorillonite = 64.4 mg g^{-1}) (Minisy et al. 2021).

Guesmi et al. (Guesmi et al. 2018) synthesized hydroxyapatite/sodium alginate nanocomposite with 5 and 10% sodium alginate content for the removal of methylene blue from the aqua medium. Polysaccharide sodium alginate, a linear polymer consisting of α -guluronic acid and 1,4- β -mannuronic acid, is a nontoxic, excellent adsorbent for organic contaminants, and biodegradable derived from alginic acid (found in the cell wall of marine algae) (Feng et al. 2017; Guesmi et al. 2018; Ragab et al. 2021). Sodium alginate has the ability to create a 3D framework hydrogel with strong hydrophobic nature owing to the abundance of -OH and -COOH groups that can cross-link with various divalent ions (i.e., Zn^{2+} , Ba^{2+} , Ca^{2+} , etc). As a result, sodium alginate may be used as a framework to create the composite hydrogel (Feng et al. 2017), the hydroxyapatite/sodium alginate nanocomposite with varying alginate content from 0 (surface area = $21.640 \text{ m}^2 \text{ g}^{-1}$; pore volume = $0.1620 \text{ cm}^3 \text{ g}^{-1}$; pore diameter = 300 \AA), 5 (surface area = $12.840 \text{ m}^2 \text{ g}^{-1}$; pore volume = $0.1147 \text{ cm}^3 \text{ g}^{-1}$; pore diameter = 357.3 \AA), and 10% (surface area = $3.390 \text{ m}^2 \text{ g}^{-1}$; pore volume = $0.02434 \text{ cm}^3 \text{ g}^{-1}$; pore diameter = 286.8 \AA). The filling of hydroxyapatite pores with alginate could be the reason for decreased surface area and pore volume of hydroxyapatite upon the incorporation of sodium alginate. The nanocomposite with 10% sodium alginate shows maximum adsorption (79.91% removal, $77.25 \text{ m}^2 \text{ g}^{-1}$ adsorption capacity) followed by 5% sodium alginate (75.25% removal, $72.69 \text{ m}^2 \text{ g}^{-1}$ adsorption capacity) and only hydroxyapatite (53.68% removal, $49.77 \text{ m}^2 \text{ g}^{-1}$ adsorption capacity). “N” of methylene blue could interact with “P-OH” functionality present on hydroxyapatite via H-bonding and “Ca-OH” group by electrostatic interaction, and as a result, methylene blue is adsorbed on the hydroxyapatite. Despite lowered surface and pore volume, the increased adsorption of methylene blue on hydroxyapatite/10% sodium alginate can be assigned to the interaction between the negative surface of COO^- present on sodium alginate in nanocomposite and positive charge on methylene blue. Adsorption of methylene blue on hydroxyapatite/sodium alginate is of exothermic nature, hence adsorption decreases with temperature rise. The plausible explanation is weakened interactions between active sites of hydroxyapatite/sodium alginate nanocomposite and methylene blue (Guesmi et al. 2018).

Xanthan gum cross-link poly(acrylic acid) hydrogel reinforced with oxidized multiwall carbon nanotubes (MWCNTs) can show potential adsorption capacity for the removal of cationic methylene blue dye. MWCNTs impart some valuable characteristics to the xanthan gum cross-link poly(acrylic acid) hydrogels such as short intraparticle distance, tunable pore size, and surface functionality, and enhanced specific surface area. In addition to that, the oxidation of MWCNTs creates more negative charge (COO^-) available on the surface of the nanocomposite,

eventuating in enhanced adsorption of cationic dyes. The negative surface on COO^- on the nanocomposite interacts with positively charge functionality on dye (i.e., $=\text{N}^+\text{H}^-$). Congo red, an anionic dye containing negative SO_3^- groups, can interact very poorly with xanthan gum cross-link poly(acrylic acid)/oxidized MWNTs, as a result the adsorption of anionic Congo red is poor compared to cationic methylene blue. Using xanthan gum cross-link poly(acrylic acid)/oxidized MWCNTs, the maximum adsorption capacity for methylene blue can reach 521.0 mg g^{-1} , which is higher than xanthan gum cross-link poly(acrylic acid) hydrogel without nanofiller (445.3 mg g^{-1}) because of the presence of more surface-active negative sites and increased specific surface area of hydrogel upon reinforcement of oxidized MWCNTs. The adsorption of methylene blue on xanthan gum cross-link poly(acrylic acid) was endothermic, i.e., removal efficiency increases with increasing temperature, but that on xanthan gum cross-link poly(acrylic acid)/o-MWCNTs changes to exothermic nature (Makhado et al. 2018).

Among transition metal oxide nanoparticles, zinc oxide (ZnO) is among the potential nanosize metal oxides favored owing to preferable biocompatibility, nontoxic property, high surface area, desirable adsorptive property, and high chemical stability. Filling nanosize ZnO within the matrix of biopolymers leads to increased reactive oxygen in the bio-nanocomposite material (Reghioia et al. 2021). The sodium alginate-based hydrogel nanocomposites can improve the adsorption of cation dyes from the wastewater, i.e., sodium alginate poly(acrylic acid) containing ZnO nanoparticles shows a maximum adsorption capacity of 1529.6 mg g^{-1} , higher than the hydrogel without nanofiller (1129 mg g^{-1}) for the adsorption of methylene blue (pH = 6, contact time = 40 minutes). The thermodynamics studies indicate the endothermic and exothermic nature of methylene blue adsorption on sodium alginate poly(acrylic acid)/ZnO and sodium alginate poly(acrylic acid) hydrogel, respectively. The authors studied the effect of sodium chloride (NaCl) salt on the adsorption performance of nanocomposite. When the competitive adsorption study using methylene blue and NaCl was carried out, it was found that the adsorption proficiency for methylene blue on sodium alginate/poly (acrylic acid) decreased slightly but decrement was high in the case of sodium alginate/poly(acrylic acid)/ZnO nanocomposite, indicating more adsorption of Na^+ on the negative surface of nanocomposite due to electrostatic interactions than methylene blue (Makhado et al. 2020).

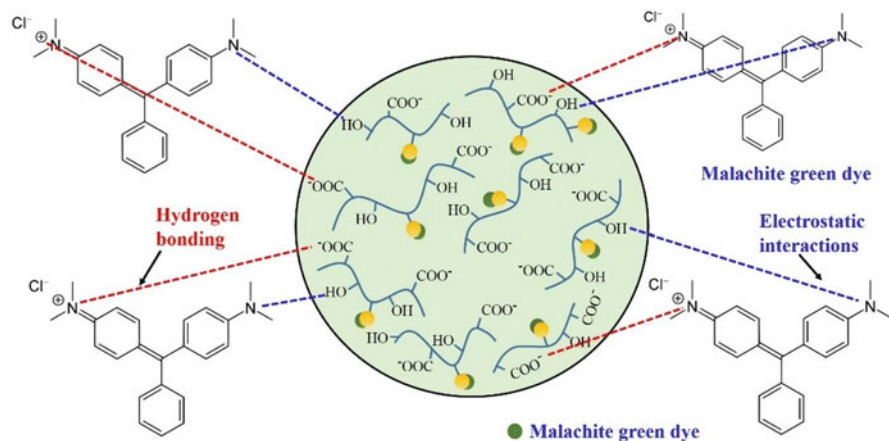
Biocompatibility, nano-range size, high specific surface area, tunable functionality, and magnetic nature of magnetite (Fe_3O_4) have opened a new path to synthesizing nanocomposites with magnetic characteristics (Nakhjiri et al. 2021). These characteristics of Fe_3O_4 can be incorporated into chitosan along with ZnO nanomaterial to yield a hybrid magnetic nanocomposite with improved adsorption capacity toward anionic dyes. Chitosan cross-linked with benzil (a diketone)/ZnO/ Fe_3O_4 nanocomposite can improve the adsorption of Remazol Brilliant Blue R anionic dye up to 620.5 mg g^{-1} . This hybrid nanocomposite material can provide various active sites for adsorption because of the presence of three components in the nanocomposite. As a consequence, various adsorbent-adsorbate interactions like H-bonding, $n-\pi$, $\pi-\pi$ interactions, and electrostatic attraction can take place resulting in the higher adsorption of anionic dye. The positive charge ($\text{Zn}(\text{OH})^+$) and positive

functionality ($-\text{NH}_3^+$, $\text{C}=\text{NH}^+$, and $-\text{OH}_2^+$) on the chitosan/benzil/ZnO/Fe₃O₄ nanocomposite allow electronic attraction with Remazol Brilliant Blue R having a negative surface because of the presence of $-\text{SO}_3^-$ ion. The presence of free “H” on chitosan/benzil/ZnO/Fe₃O₄ nanocomposite allows H-bonding interaction with negative atoms (i.e., O and N) of Remazol Brilliant Blue R dye. Another type of H-bonding (Yoshida H-bonding) between aromatic moiety of dye and -OH functional groups of chitosan/benzil/ZnO/Fe₃O₄ is also possible. In addition to these, the π - π interaction between aromatic moieties of dye and chitosan/benzil/ZnO/Fe₃O₄ (from benzil and aromatic diketone) and n- π interaction between lone pairs of N and O and aromatic ring of dye make a possible enhancement in the adsorption of Remazol Brilliant Blue R on chitosan/benzil/ZnO/Fe₃O₄ compared to any of the individual constituents (Reghioua et al. 2021).

Guar gum-graft-poly(acrylamide)/silica nanocomposite impart a good adsorption capacity for anionic dyes such as reactive blue and Congo red. Intramolecular hydrogen bonding and the hydrophobic nature of Guar gum results in poor adsorption sites. The limitation can be solved by grafting polyacrylamide onto Guar gum, the grafting results in new hydrogel having more -OH functionality and an additional amide ($-\text{CONH}_2$) functionality resulting in better interaction with dye. Further addition of SiO₂ creates additional -OH functionality in the nanocomposite, improves hydrodynamic volume, and provides a larger active specific surface area for the adsorption. For reactive blue, a maximum adsorption capacity of 579.01 mg g⁻¹ was achieved within 40 minutes and in the case of Congo red, maximum adsorption of 233.24 mg g⁻¹ was achieved within 30 minutes. The zeta potential study indicates that the Guar gum-graft-poly(acrylamide)/silica nanocomposite has a positively charged surface (pH = 4.48). Hence, at pH below 4.48, the protonation of Guar gum-graft-poly(acrylamide)/silica nanocomposite takes place leading to a positive surface which may interact more efficiently with the negative surface ($-\text{SO}_3^-$) of dye because of opposite charges resulting in good adsorption of Guar gum-graft-poly(acrylamide)/silica nanocomposite toward anionic Congo red and reactive blue dyes. Formation of outer-sphere surface complex results in a decrement in the adsorption of anionic dyes with increasing ionic strength using salts, i.e., NaCl and Na₂SO₄. The complex formation is a result of the transfer of anions at the site of adsorption arising from hydrogen bonding within nanocomposite and anion of salt. Owing to the higher screening effect of SO_4^{2-} anion than Cl^- ion, the presence of SO_4^{2-} anion results in a greater decrement in the anionic dye adsorption (Pal et al. 2015).

Gum arabic (highly branched heteropolysaccharide obtained from acacia tree) cannot be utilized in a few applications in its original state due to flaws including unregulated hydration process and microbial contamination, loss in viscosity on stockpiling, and thickness; consequently, for most situations, it is bonded with other monomer units (i.e., acrylamide) to address these flaws. The main mechanism of adsorption on gum arabic nanocomposites is governed by pore diffusion, H-bonding, and electrostatic interactions. A comparative study on the removal efficiency of gum arabic cross-links poly(acrylamide)/ZnO nanocomposite shows that the nanocomposites exhibit greater adsorption for cationic dye (malachite green) than the anionic dye (methyl orange) owing to the additional electrostatic attraction between

gum arabic cross-link poly(acrylamide)/ZnO composite and malachite green. The competitive adsorption studies impart that removal efficiency for cationic dyes, i.e., methylene blue and malachite green can be reached >60%, double that for anionic dyes, i.e., methyl orange and Congo red (<30%), indicating preferential adsorption of cationic dyes over anionic dyes on gum arabic cross-link poly(acrylamide)/ZnO nanocomposite. In the mixture of five portions of cationic dye malachite green and one portion of anionic dye methyl orange, the maximum adsorption capacity of the gum arabic cross-link poly(acrylamide)/ZnO nanocomposite reach 766.52 mg g⁻¹. The regeneration study suggests that gum arabic cross-link poly(acrylamide)/ZnO nanocomposite can be used for up to seven cycles with no performance loss and after that mere 4% reduction in the adsorption performance was observed on the tenth reuse cycle. Because of the structural framework, gum arabic cross-link poly(acrylamide)/ZnO nanocomposite is immensely anionic at surface charge <pH owing to the numerous anionic binding sites (i.e., functionality like -COO⁻ and -OH) in gum arabic and hence there is a high probability for binding of cationic dyes than anionic dyes via electrostatic interactions (Scheme 1) and H-bonding with -OH groups present on the surface of ZnO nanofiller of the nanocomposite. The electrostatic attraction of cationic dye on nanocomposite can be confirmed by decreased adsorption of cationic dyes on the nanocomposite in the presence of cations like Ca²⁺ and Na⁺. The polar “N” atom of malachite green dye might result in hydrogen bonding with carboxyl group, but electrostatic interactions will dominate the H-bonding, and, therefore, H-bonding involvement would not impact much. The nanocomposite hydrogel possesses porous structure and hence diffusion of malachite green within the pores of composite



Scheme 1 Proposed mechanism for the adsorption of methylene blue onto gum arabic cross-link poly(acrylamide)/ZnO. (Reprinted by permission from [Mittal et al. 2020]; Springer; Journal of Polymers and the Environment; In Situ Synthesis of ZnO Nanoparticles Using Gum Arabic-Based Hydrogels as a Self-Template for Effective Malachite Green Dye Adsorption; Hemant Mittal et al. copyright [2020])

material could also possibly contribute to its adsorption on gum arabic cross-link poly (acrylamide)/ZnO (Mittal et al. 2020).

The adsorption of cationic dyes methyl violet and crystal violet can significantly improve by using β -cyclodextrin-based nanocomposites. β -cyclodextrin is composed of seven glucopyranose units arranged in a cyclic structure via glycosidic bonds. The arrangement of these glucopyranose units gives β -cyclodextrin a cone shape appearance in which the outer part acts as a hydrophilic and the inner part as a hydrophobic site. Hence, β -cyclodextrins' inner cavity has the capability to act as a host for various organic molecules (Heydari et al. 2018; Rout and Jena 2022). Therefore, the addition of β -cyclodextrin to various polymeric macromolecules results in increased adsorption of the material, i.e., hydroxypropyl- β -cyclodextrin–polyurethane nanoconjugates containing magnetic Fe_3O_4 nanofiller have a maximum adsorption capacity of 1667 and 1269 mg g^{-1} for cationic dyes methyl violet and crystal violet, respectively. Hydroxypropyl- β -cyclodextrin–polyurethane adsorbent demonstrated great adsorption efficiency for the removal of both methyl violet as well as crystal violet from the synthetic waste aqueous solution and therefore has a great potential to act as a promising adsorbent material for the proficient adsorption of cationic dyes in an aqueous medium (Nasiri and Alizadeh 2019). Another potential adsorbent for high adsorption of methylene blue is sodium alginate cross-link polyacrylic acid hydrogel reinforced by TiO_2 . It has a very high maximum adsorption capacity for methylene blue, 2257.36 mg g^{-1} . The content variation in TiO_2 nano-reinforcement suggests improved adsorption efficiency of the nanocomposite for the methylene blue. The plausible explanation is that increased TiO_2 content results in increased surface-active site and increased negative charge on the surface allows more interaction between the nanocomposite and the cationic dye. The growth in absorption of methylene blue may be attributable to charge-repulsive force inside the gels' cross-link framework. The negative surface of the TiO_2 pushes away the $-\text{COO}^-$ ion because of similar charge. This leads to increased available surface-to-volume ratio of nanocomposite on which the positively charged methylene blue can attach itself. The 80% removal efficiency (for methylene blue) of sodium alginate cross-link polyacrylic acid gel was increased to 99.4% by reinforcement of a mere 2% TiO_2 . The effect of enhanced adsorption of methylene blue on nanocomposite with only 2% TiO_2 could have been observed because of the large specific surface area (186 $\text{m}^2 \text{g}^{-1}$) of TiO_2 than other inorganic clay nanofillers. The mechanism of adsorption was governed by electrostatic attraction between the positive surface of methylene blue and the negative surface of composite owing to $-\text{COO}^-$ and H-bonding between imine of methylene blue and $-\text{OH}$ of nanocomposite (from TiO_2 surface) (Thakur et al. 2016).

Pesticides

Pesticides are substances that are used in the agro sector to exterminate, expel, or suppress pests such as fungus, rats, bugs, and grasses. If allowed to thrive unchecked in agricultural land masses, these pests could be damaging or cause postharvest

losses. As a result, pesticides are popular for disposing of or controlling pests in agricultural regions, enhancing agricultural output, and, as a result, raising the global public's livelihood. Annually, almost two million tonnes of pesticides are used to combat insects, parasites, and unnecessary vegetation across the world. Pesticides are categorized as rodenticides, fungicides, insecticides, herbicides, and so on, depending on the specific target that is required to be eliminated. Herbicides and insecticides are the most well-known and commonly used pesticides, accounting for 29.5% and 47.5% of overall pesticide usage, respectively (Rana et al. 2021). The growing human population requires more resources to sustain life leading to increasing demands for agricultural products. In addition to that, the storage of such food also requires the elimination of pests to reduce agricultural loss. Therefore, the use of pesticides is continuously increasing. Pesticides can easily become a part of the food chain through soil or water. Pesticides are hazardous and cannot be easily degraded. Therefore, it is a necessity to eliminate pesticides from the water through proper treatment. The following are some types of pesticides classified according to their application type:

Herbicides: Used to prevent unnecessary vegetation in the crop, i.e., the unwanted weeds in the crop may compete with the crop for nutrients leading to decreased crop yield, e.g., atrazin, butachlor, simazine, ametryn, etc.

Fungicides: Inhibits fungi growth, e.g., chloranil, dichloran, hexachlorobenzene, captofol, etc.

Insecticides: Eliminates/Control insects, e.g., toxaphene, parathion, phenylpyrazole, etc.

Rodenticides – To eliminate rats and mice, e.g., warfarin, sodium fluoroacetate, α -naphthylthiourea, etc.

Butachlor, N-(butoxymethyl)-2-chloroN-(2,6-diethylphenyl) acetamide is among the most widely used herbicides in Asia during rice and wheat production to reduce undesired vegetation and periodic grasses with a yearly consumption rate of 45 thousand tonnes because of its minimal cost and diverse efficacy. It suppresses the lengthening of the chain fatty acids and key enzymes in geranylgeranyl pyrophosphate cyclization. Butachlor's unregulated use has resulted in environmental concerns due to erosion, weathering, and drainage of water, posing a threat to public health and marine life forms. It has already been found in surface water, soil, and groundwater, with remnant quantities ranging from 100–1400 ng L⁻¹ in fresh water, and 29,500–74,000 ng kg⁻¹ in soil because of agricultural usage. Owing to its lipophilic nature, it is nonresponsive to natural deterioration and tends to accumulate in the body of aquatic creatures, creating a variety of hazardous consequences. Butachlor was found to be a potential carcinogenic in toxicology investigations on many marine creatures, causing anatomical defects, inhibiting egg development, and causing considerable fatality in zebrafish *Danio rerio*. It has the tendency to create oxidative toxicity and cytotoxicity in fish by inhibiting the glutathione detoxification system in the kidney, as well as affecting zebrafish's reproductive function. Butachlor has been shown to be mutagenic to frogs, a development suppressant in earthworms, and a possible tumor enhancer with cancerous effects in mice livers.

Butachlor lacks the functional group having the capability to hydrolyze and, therefore, it cannot be hydrolyzed naturally (Kaur et al. 2021).

Chitosan/Poly(vinyl alcohol) hydrogel reinforced with Ag nanoparticles has a capacity to adsorb butachlor from the aqueous medium. Ag nanoparticle deposition in the polymer matrices improves the density of cross-linking leading to a larger surface-to-volume ratio and the formation of nanoscale pores in the composite material, i.e., the specific surface area of chitosan/poly(acrylic acid) gel increases from 6.579–8.316 m² g⁻¹, pore volume increases from 0.011–0.016 cm³ g⁻¹, and pore diameter increases from 1.98–2.33 nm upon nanofilling of polymer matrix with Ag nanoparticles. The actual surface area of the gel could potentially be higher than these values for the powdered sample because of swelling. The enhanced textural parameters of chitosan/poly(acrylic acid)/Ag nanocomposites impart a potential for better adsorption than chitosan/poly(acrylic acid) gel. The adsorption studies suggest that the maximum adsorption capacity of butachlor on chitosan/poly(acrylic acid)/Ag nanocomposites was 23.81 mg g⁻¹, slightly higher than chitosan/poly(acrylic acid), suggesting a possible contribution of Ag in the adsorption performance. The mechanism contributing significantly to butachlor sorption is thought to involve electrostatic attraction (H-bonding) and pi-pi interaction. The mechanism contributing significantly to butachlor sorption is thought to involve π - π (between chitosan and the aromatic moiety of butachlor) and electrostatic interactions. At lower pH, -NHCO- functional group in chitosan/poly(acrylic acid)/Ag is protonated and, therefore, the number of sites for H-bonding is increased. On the other hand, an increase in pH does not allow the protonation of “-NHCO-” group leading to a decrement in sites for H-bonding on the surface of the nanocomposites, consequently the adsorption of butachlor is reduced. The removal efficiency of chitosan/poly(acrylic acid) was reduced to 71.06% between the first and fifth reusability cycle and that of chitosan/poly(acrylic acid)/Ag nanocomposites were reduced to 79.32%, suggesting good adsorption efficiency of nanocomposites after the fifth cycle compares to hydrogel without nanofilling. This reduction in sorption is most likely attributable to the saturation of active adsorption sites owing to the difficulty of butachlor to desorb from these sites (Kaur et al. 2021).

Exposure to methyl parathion pesticide, a frequent contaminant found in farming untreated wastewater, causes organophosphate poisoning along with lethal conditions characterized by confusion, sweating, migraine, diarrhea, convulsions, etc., hence it is required to dispose water into the streams after proper treatment. The maximum methyl parathion pesticide adsorption capacities achieved with bentonite incorporated in chitosan and (chitosan and 2-hydroxyethyl methacrylate/methyl methacrylate) polymers are 634.5 and 868.5 mg g⁻¹, respectively, against 287.3 mg g⁻¹ maximum adsorption capacity of bentonite clay alone. The better performance of bentonite/chitosan than bentonite/(chitosan and 2-hydroxyethyl methacrylate/methyl methacrylate) could be due to a larger surface area and smaller pore diameter of the latter (e.g., surface area and pore volume of bentonite/chitosan was 106.4 m² g⁻¹ and 14.6 nm, respectively; for bentonite/(chitosan and 2-hydroxyethyl methacrylate/methyl methacrylate) surface area was 118.2 m² g⁻¹ and pore diameter was 12.6 nm). This may have been connected to polymeric

networks' participation in the formation of secondary microspores, which increase the porosity and surface area of the formations. As bioadsorbents for methyl parathion pesticide, all compositions, i.e., bentonite, bentonite/(chitosan and 2-hydroxyethyl methacrylate/methyl methacrylate), and bentonite/chitosan have a high recovery and recycling efficiency. The bentonite can remove >86 and > 81% of pesticide after the third and fifth recycle periods. The nanocomposite bentonite/chitosan was able to maintain a 100% adsorption efficiency for the second cycle and slightly declined to 95% in the fifth recycle period. The bentonite/(chitosan and 2-hydroxyethyl methacrylate/methyl methacrylate) adsorbent shows a decline in removal percentage to 97% after the fifth cycle, suggesting potential recyclability capabilities for commercial usage of the biopolymer nanocomposites as an adsorbent for methyl parathion pesticide removal from wastewater (Abukhadra et al. 2021).

The pesticides containing wastewater discharge do not only have a single pollutant in them. The presence of other pollutants could influence the adsorption capacity of the adsorbent in real applications. The most common ions are ammonium (NH_4^+) and phosphate (PO_4^{3-}) because of their use as fertilizers. A study of adsorption of methyl parathion pesticide in the presence of PO_4^{3-} , NH_4^+ , Mn^{2+} , and Pb^{2+} was carried out by Abukhadra et al. (Abukhadra et al. 2021). The adsorption of methyl parathion on bentonite significantly declined to ~50% in the presence of Mn^{2+} ions and between 27 and 34% in the presence of NH_4^+ , Pb^{2+} , and PO_4^{3-} ions. The substantial decline in pristine bentonites' selectivity for methyl parathion in the presence of these ions was reasonable owing to bentonites' high cation exchange characteristics and preference for inorganic cations over organic pesticides. The adsorption affinities of both bentonite/chitosan and bentonite/(chitosan and 2-hydroxyethyl methacrylate/methyl methacrylate) for methyl parathion were considerably declined with the presence of PO_4^{3-} and Pb^{2+} ions and marginally influenced by the presence of NH_4^+ and Mn^{2+} ions, still the modified nanocomposite adsorbent manages to provide good adsorption for methyl parathion (Table 2). This can be attributed to the transformation of clay into an adsorbent with preferential adsorption of organic moiety over inorganic ions during the intercalation. Therefore, the biopolymer addition in bentonite clay improves the adsorption efficiency as well as selectivity for the adsorption of methyl parathion from the synthetic wastewater.

Three mechanisms influence the adsorption reactions of adsorbents: (i) electrostatic interaction, (ii) ion exchange, and (iii) chemical complexation. In

Table 2 Effect of various ions on the removal efficiency of clay-biopolymer-based nanocomposite for methyl parathion pesticide (data obtained from [Abukhadra et al. 2021])

Adsorbent	% removal of methyl parathion on adsorbent in the presence of ions			
	NH_4^+	PO_4^{3-}	Mn^{2+}	Pb^{2+}
Bentonite	33.4	27.8	50.3	30.6
Bentonite/Chitosan	80.3	56.5	86.7	64.8
Bentonite/(Chitosan and 2- hydroxyethyl methacrylate/ methyl methacrylate)	78.6	53.5	84.3	57.2

nanocomposites, hydroxyl attached to Al and Si in clay minerals facilitates electrostatic interaction and complex formation (Abukhadra et al. 2021).

Other chitosan-based nanocomposites filled with zeolite provide the composite with efficient active surface functionality, increased stability and reactivity, and biodegradability along with enhanced adsorption for organic compounds. Mostafa et al. studied the adsorption of chitosan/zeolite nanocomposite to explore its possible adsorption for three organophosphorous pesticides, namely methyl parathion, acephate, and omthosate (Mostafa et al. 2021). The eco-friendly chitosan/zeolite nanocomposites display an excellent adsorption capacity for all three pesticides. The adsorption capacity for methyl parathion, acephate, and omthosate can be reached 560.8, 650.7, and 506.7 mg g⁻¹, respectively, owing to increased surface functionality, specific surface area, and pore volume in chitosan/zeolite nanocomposite (446.7 m² g⁻¹ surface area, 23.6 nm pore diameter, and 0.412 cm³ g⁻¹ pore volume) than zeolite alone (423.0 m² g⁻¹ surface area, 11.6 nm pore diameter, and 0.382 cm³ g⁻¹ pore volume) (Mostafa et al. 2021).

The competitive adsorption studies of these pesticides in the presence of ions like Cd²⁺, Zn²⁺, Pb²⁺, PO₄³⁻, and NO₃⁻ are given in Table 3 (Mostafa et al. 2021). Although the removal efficiency of chitosan/zeolite for pesticides declined greatly, the decline in the removal efficiency was higher in the presence of PO₄³⁻ among PO₄³⁻ and NO₃⁻ anions. That might be due to the great preferences of adsorbents for PO₄³⁻ ion to make a variety of combinations with the -OH of both zeolite and chitosan as well as their interchangeable characteristics with the ionic constituents of clay. The least effect was observed in the presence of Cd²⁺, indicating the preferential adsorption of organic pesticides over Cd²⁺ on chitosan/zeolite nanocomposite (Mostafa et al. 2021). The application of the chitosan/zeolite composite in real wastewater may further decline the adsorption as other pollutants among themselves for the active adsorption sites of the adsorbent.

The chitosan/zeolite adsorbent can be used for up to five recycle periods with minimum loss in the removal efficiency, i.e., ~95% for methyl parathion, 95% for acephate, and ~92% for omthosate (Mostafa et al. 2021).

Unlike dyes, the adsorption of pesticides is not highly efficient. However, some adsorbent may adsorb pesticides more preferentially over dyes, i.e., cadmium sulfide (Cds) reinforced with silanized cellulose nanofibers can preferentially adsorb

Table 3 Competitive adsorption of methyl parathion, acetaphate, and omthosate pesticides on chitosan/zeolite nanocomposites in the presence of anions and cations (data obtained from [Mostafa et al. 2021])

Ions	Removal efficiency (%)		
	Methyl parathion	Acephate	Omthosate
Cd ²⁺	80.7	83.4	77.8
Zn ²⁺	61.3	67.5	55.7
NO ₃ ⁻	52.4	60.3	48.7
PO ₄ ³⁻	46.2	52.7	34.7
Pb ²⁺	45.8	47.3	40.2

chlorpyrifos pesticide (86.96 mg g^{-1}) over coloring pigments methylene blue (26.66 mg g^{-1}) and safranin-O (17.86 mg g^{-1}). Adsorbent performance is greatly attributed to the increased silanized cellulose nanofibers content, with peak adsorption reaching for Cds incorporated with 10% silanized cellulose nanofibers, and then a decrement in adsorption characteristics was noticed, attributed to increased constraint for efficacious CdS nucleation and altered responsiveness of effective surface area for contaminant species. This is a financially viable and commercially exploitable substance for pollutants removal due to its uncomplicated method for synthesis, bio-based fruitful adaptation, and accelerated sorption attributes (Komal et al. 2020).

In recent years, triazine herbicides have been frequently utilized to suppress weeds in a range of agricultural crops. Such pesticides are essential in the production of grains like rice and maize. Their remnants, on the other hand, are becoming a major cause of pollutants in the environment. Triazine herbicides may potentially pose a risk to humans, including birth abnormalities, disruption of hormone systems, and cancer. The variations between the six triazine insecticides adsorbed by the cellulose/reduced GO nanocomposite must be exploited by comprehending how the adsorbates interact with the nanocomposite. The triazine insecticides' strong bonding structure, as well as the electron releasing properties of the O, N, and S atoms, can help with adsorption. All six triazine insecticides are structurally related and contain nearly the same number of nitrogen atoms. As a result, most pesticides' π -bonding networks are remarkably similar. As a result, the discrepancies might be due to the O, Cl, and S atoms, as well as van der Waals interactions. Comparing the structure of ametryn, prometryn, and simeton, the "O" atom in simeton differs from the "S" atoms in ametryn and prometryn in addition to the additional branch in ametryn and prometryn (Fig. 2). Ametryn has a higher potential for contributing electrons since it includes a "S" which has a lower electronegativity compared to "O" atom. Therefore, the adsorption of ametryn on cellulose/reduced GO adsorbent is higher. On the other hand, despite the presence of "S," prometryn contains an extra branched structure that impairs van der Waals interactions between adsorbent and

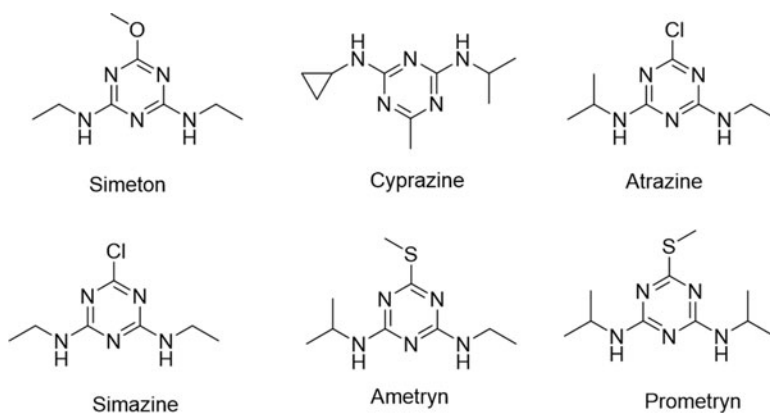


Fig. 2 Structure of triazin pesticides

prometryn, making it the least adsorptive of the triazine insecticides. The “Cl” atoms of atrazine and simazine are electron-withdrawing because of high electronegativity. “Cl” atoms may provide an acidic π -system that quickly forms complexes with the π -system with no derivative and hence aids the adsorption. Surface studies implicate rough and wrinkled surfaces with a lamellar structure which is a favorable situation and significantly boosts the adsorption of the nanocomposite. The proposed nanocomposites could be recycled with 85% adsorption efficiency up to the sixth cycle (Zhang et al. 2015).

Neonicotinoid pesticides (e.g., imidacloprid, acetamiprid, dinotefuran, etc.) are a type of diverse fast insecticides that have been used worldwide to eradicate bugs. They are indeed the main substitutes for carbamates and organophosphorus. As a result, neonicotinoid pesticides serve an important part in the preservation of a wide range of crops, including rice, fruits, maize, and vegetables. Neonicotinoid contaminants can contaminate natural waterways, vegetables/fruits, and soil as a consequence of their widespread usage and buildup over time, posing significant health and ecological concerns (Liu et al. 2017). In nanocomposites, a highly porous network of cross-linked polymers can provide better removal efficiency for both organic and inorganic pollutants. The porous polymeric structure based on a metal-organic framework includes a strong bridging/chelating between a metal ion and an organic moiety. Metal-organic framework based on magnetic $\text{Fe}_3\text{O}_4/\text{GO}/\beta\text{-CD}$ nanocomposite core and Cu-benzene-1,3,5-tricarboxylate with high specific surface area ($250.33 \text{ m}^2 \text{ g}^{-1}$) and pore volume ($0.826 \text{ cm}^3 \text{ g}^{-1}$) could adsorb neonicotinoid from the aqua medium. The nanocomposites-based porous metal-organic framework has a maximum adsorption capacity ranging between 1.77 and 3.11 mg g^{-1} for a variety of neonicotinoids. The findings indicate that the established metal-organic framework was a simple and suitable adsorbent for removing neonicotinoid pesticides from the aquatic ecosystem (Liu et al. 2017).

Pharmaceutical Drugs

Medications have been regarded as emergent contaminants in the marine ecosystem over the past few decades owing to their long persistence, limited degradability, and negative impacts on animal and human health as well as marine life. Medical drug companies, sanitary items, health care effluent, and excretion via feces/urine are all major sources of pollution in water. While the quantity of pharmacological untreated wastewater discharged into the environment is small, the discharge of this sort of contaminant into the environment may happen and represent a long-term hazardous danger to freshwater bodies and life forms. The antibiotics are most frequently used therapeutically active drugs. The increased use of these antibiotics has led to their accumulation in fresh water, contaminating the water (Gor and Dave 2020; H. Ragab et al. 2021; Karimi-Maleh et al. 2021; Luo et al. 2019). Third-generation fluoroquinolones drug ciprofloxacin is antibacterial with a water solubility of 11.48 g L^{-1} . Ciprofloxacin concentrations in hospital discharge range between 0.7×10^{-6} and $1.24 \times 10^{-4} \text{ g L}^{-1}$ and it is hazardous to some microbes at extremely

low concentrations. As a result, ciprofloxacin elimination from untreated water is critical. Nickel ferrite (NiFe_2O_4), a magnetic nanomaterial that can be reinforced into modified gum ghatti cross-link poly(acrylamide) hydrogel can effectively remove ciprofloxacin with a maximum adsorption capacity of 274.84 mg g^{-1} (Gor and Dave 2020). The hydrophobic interaction (antibiotic and the oxygen-containing moiety of nanocomposites), $n-\pi$ interactions, and H-bonding are all involved in gum ghatti/poly(acrylamide)/ NiFe_2O_4 nanocomposites' removal of ciprofloxacin (Gor and Dave 2020).

Tylosin is a common animal health antimicrobial drug that has been shown to facilitate the development in animals and is extensively used as a pharmacotherapeutic medicine in the diagnosis of mycoplasmosis in livestock and poultry. Aggregated tylosin might cause gene problems, raise bacterial resistance to antibiotics, and adversely affect human and aquatic life forms (Luo et al. 2019). Porous charcoal derived from carbon-containing sources can contribute significantly to adsorption, i.e., polymeric matrix may be benefited from its broad and regulated pore structure and size, large surface-to-volume ratio, excellent stability, and adsorption efficiency. Moreover, highly porous charcoal may be produced using less expensive resources such as biomass waste (Mashile et al. 2020). Activated charcoal reinforced into chitosan/cellulose matrix shows potential for the removal of tylosin having a maximum adsorption capacity of 59.26 mg g^{-1} . The adsorption of tylosin on the nanocomposite is controlled by $\pi-\pi$ and electrostatic interactions (repulsion between protonated tylosin and the positive surface of adsorbent at $\text{pH} < 7.2$), H-bonding (between surface $-\text{OH}$ and $-\text{COOH}$ groups of nanocomposite and tylosins), internal diffusion, and size exclusion impact. The study of kinetics revealed that tylosin sorption on chitosan/cellulose/activated charcoal is a sluggish phenomenon. The 23.844 nm pore size of the nanocomposite suggests a mesoporous adsorbent. Tylosin has a flexible structure and hence can diffuse into the adsorbents' pores. As a result, the size exclusion effect affects tylosin adsorption by nanocomposite (Luo et al. 2019).

The specific substance having a magnetic property may be simply retrieved by an outer magnetic force positioned on the exterior of the vessel after the adsorption process is completed, with no extra centrifugation or filtering of the specimen. As a result, magnetic separation is straightforward, a less expensive, and faster way of separating such tiny nanocomposite particles than a laborious filtration procedure (Nakhjiri et al. 2021; Nezhadali et al. 2021). The nanoparticles tend to be agglomerated and as a consequence, the adsorption of the magnetic nanomaterial is reduced. The reinforcement of magnetic nanomaterials not only enhances the dispersion and stabilized these nanomaterials, but also enhances surface functionality, which is a very critical parameter to decide adsorbents' performance (Feng et al. 2017; Nezhadali et al. 2021). Carbamazepine (treatment of nerve pain and epilepsy) is persistent in the environment. Carbamazepine can be removed from the industrial effluents using polypyrrole-chitosan/ Fe_3O_4 magnetic nanocomposite. The maximum adsorption capacity of polypyrrole-chitosan/ Fe_3O_4 was 121.95 mg g^{-1} with a slight loss in performance ($<5\%$) after recycling and reusing it for the fifth cycle. The polypyrrole-chitosan/ Fe_3O_4 can remove 94.5% carbamazepine from the

aqueous medium within 25 minutes. In addition to that the presence of magnetic characteristics makes its separation from aqueous solution a lot more efficient. Hence, polypyrrole–chitosan/ Fe_3O_4 is a better nanocomposite that can be used with enhanced adsorption compared to only Fe_3O_4 nanoparticles (64% removal efficiency) for carbamazepine adsorption. Carbamazepines' pKa value (~ 13.9) suggests it possesses a neutral structure between 2 and 9 pH value. In a similar range, polypyrrole moiety of nanocomposite may have a cationic charge, but as carbamazepine do not possess any charge in the pH range in which polypyrrole-chitosan/ Fe_3O_4 has a charged structure, therefore, there are no electrostatic interactions between adsorbate and adsorbent. H-bonding, dipole-dipole, and π - π interactions between adsorbate and adsorbent governs the adsorption of carbamazepine on polypyrrole–chitosan/ Fe_3O_4 nanocomposite (Nezhadali et al. 2021).

The most frequent endocrine disruptive substances are steroids. Among the most potent endocrine-disrupting chemicals is estradiol. Estradiol and its metabolites are commonly discovered in marine ecosystems and can cause serious harm (Li et al. 2020). Chitosan reinforced with hybrid Al_2O_3 -hydroxyapatite (chitosan/ Al_2O_3 -hydroxyapatite) can efficiently remove estradiol from water. The chitosans' low surface area ($0.908 \text{ m}^2 \text{ g}^{-1}$) and pore volume ($0.003 \text{ cm}^3 \text{ g}^{-1}$) result in its lower adsorption of estradiol on chitosan (maximum adsorption capacity = 29.04 mg g^{-1}) despite the presence of a large number of active functional groups on its surface ($-\text{NH}_2$, $-\text{OH}$). Improvement in the textural property of chitosan by producing chitosan/ Al_2O_3 -hydroxyapatite resulted in increased adsorption of chitosan toward estradiol, i.e., the resulting chitosan/ Al_2O_3 -hydroxyapatite composite possesses improved adsorption capacity (39.78 mg g^{-1}) for estradiol owing to the increased surface $-\text{OH}$ groups, surface area ($94.823 \text{ m}^2 \text{ g}^{-1}$), and pore volume ($0.2000 \text{ cm}^3 \text{ g}^{-1}$). In addition to that, the chitosan/ Al_2O_3 -hydroxyapatite adsorbent is quite selective toward only organic pollutants. The presence of inorganic constituents did not alter the adsorption of estradiol on the nanocomposite. However, the presence of organic contaminants does affect the adsorption of estradiol (Li et al. 2020). Chitosan/ Al_2O_3 -hydroxyapatite can be used to successfully remove estradiol in the wastewater in the presence of high inorganic ion concentrations.

Because of amoxicillin's excellent resistance to pathogens and broad spectrum against a variety of pathogens, it is among the most extensively used commercialized penicillins. It is prevalent in effluents from the pharma sector and clinics, where it induces skin problems and bacterial resistance in pathogenic microorganisms. Between 30 and 90% of the total amoxicillin is emitted into the water via human and animal feces. Another drug, nonsteroidal anti-inflammatory drug, diclofenac is widely used across the globe. It is well known to get discharged into waterways in the form of potassium or sodium salt (H. Ragab et al. 2021). Limestone-activated carbon-sodium alginate shows prominent adsorption for both amoxicillin and diclofenac with a maximum removal capacity of 249 and 246 mg g^{-1} , respectively. The effect of pH suggests an electrostatic interaction must have influenced the adsorption of both drugs on nanocomposite, i.e., at lower pH (acidic pH = ~ 2), the negatively charged drugs must be attracted to protonated positive sites of the nanocomposite adsorbent, leading to an increased removal percentage of amoxicillin

(99.6%) and diclofenac (98.4%). The same can be concluded from the large decline in the removal efficiency of the nanocomposite in basic conditions because the deprotonated negative surface of drugs and limestone-activated carbon-sodium alginate repel each other (H. Ragab et al. 2021).

Mashile et al. (Mashile et al. 2020) reported the adsorption of three fluoroquinolones (antibacterial drugs), namely enrofloxacin, levofloxacin, and danofloxacin on a biodegradable magnetic mesoporous carbon/ β -cyclodextrin-chitosan. The insertion of magnetic nanoparticles into mesoporous carbon eases separation, and functionalizing the material allows a decrease in its hydrophobicity, i.e., the starch-based mesoporous carbon was having a very high specific surface area and pore volume of $1181 \text{ m}^2 \text{ g}^{-1}$ and $2.54 \text{ cm}^3 \text{ g}^{-1}$, respectively. The reinforcement of this mesoporous carbon led to magnetic-carbon/ β -cyclodextrin-chitosan nanocomposite having an excellent specific surface area and pore volume of $1264 \text{ m}^2 \text{ g}^{-1}$ and $4.65 \text{ cm}^3 \text{ g}^{-1}$, respectively. The removal of all three fluoroquinolones drugs by the magnetic-carbon/ β -cyclodextrin-chitosan nanocomposite was governed by surface adsorption as well as intramolecular diffusion of drugs within the nanocomposite. For enrofloxacin, levofloxacin, and danofloxacin the nanocomposite shows maximum adsorption of 195, 165, and 130 mg g^{-1} , respectively. These capacities were reduced to 122, 116, and 88 mg g^{-1} , respectively, on the eight regeneration-reuse cycles, imparting prominent adsorption of magnetic-carbon/ β -cyclodextrin-chitosan nanocomposite after so many reuse cycles (Mashile et al. 2020).

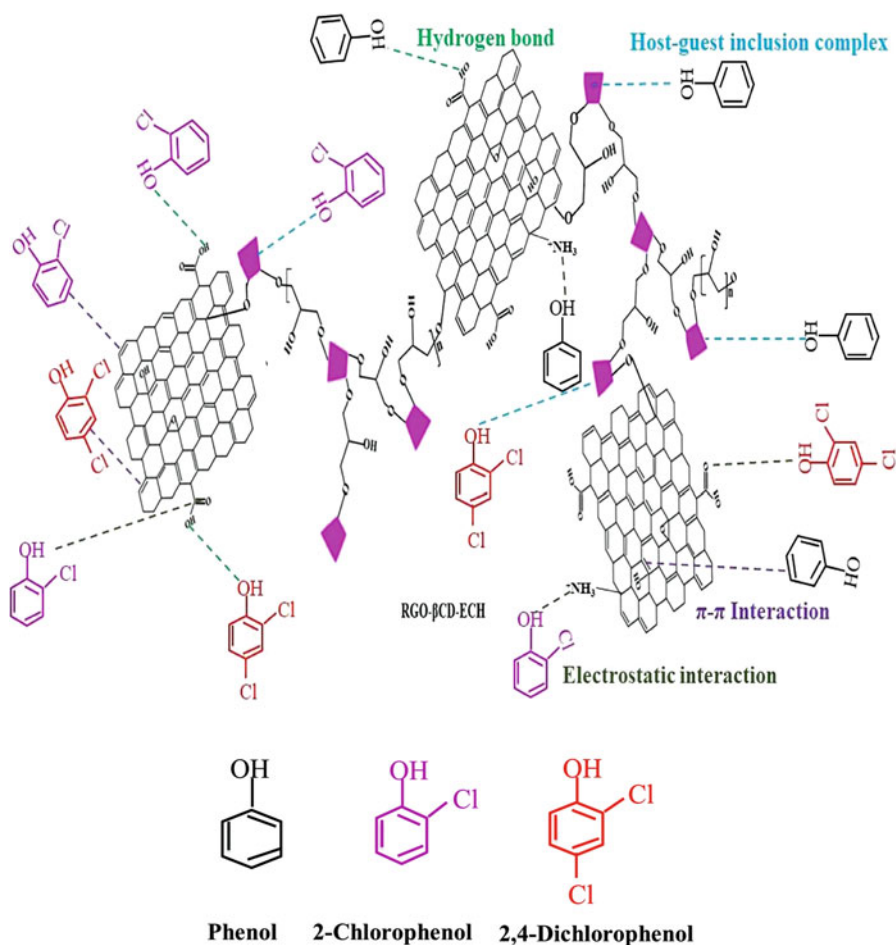
Phenols

Phenol manufacturing businesses, like pesticides, fertilizer, dye manufacturing, petroleum refineries, and so on, produce phenol constituents in their wastes. Phenols exert substantial negative impacts on health including pancreatic, protein, harm kidney and liver, cause tissue deterioration, as well as paralyze the nervous system. Migraines, vision discomfort, and high blood pressure are all side effects of phenol exposure (Fathy et al. 2020; Guo et al. 2019; Nakhjiri et al. 2021). The creation of biocompatible and hydrophilic (3D) reduced graphene oxide (rGO) hybrid hydrogels can effectively avoid rGO sheet aggregation and restacking while also broadening the spectrum of rGO-based composites in potential implementation. Reinforcement of rGO sheets into sodium alginate can help to reduce the aggregation of rGO as well as modify rGOs' hydrophilicity (Feng et al. 2017). The use of rGO/sodium alginate composition shows more adsorption for phenol (adsorption capacity = 26.126 mg g^{-1}) than bisphenol-A (adsorption capacity = 14.123 mg g^{-1}), which is quite low compared to other composite materials despite the large specific surface area of rGO ($2630 \text{ m}^2 \text{ g}^{-1}$), but better than sodium alginate which merely shows capability for phenol adsorption. The π - π interaction and H-bonding are thought to be responsible for the adsorption of the phenolic compound on rGO/sodium alginate (Feng et al. 2017).

The utilization of epichlorohydrin cross-linked β -CD polymer reinforced with GO can remove the α -naphthol and β -naphthol with a removal efficiency ranging

between 50 and 60% for the former and 60 and 70% for later. This was quite high compared to β -CD/GOs' adsorption efficiency for phenol 15–20%. The adsorption was plausibly a combined effect of both components in β -CD/GO nanocomposite. The removal efficiency of β -CD/GO for phenols was lower than GO but higher than β -CD, indicating reinforcement of GO has benefited β -CD's adsorption efficiency for phenols. The same can be verified by the fact that increasing GO content from 0–2.4 results in increased adsorption. The adsorption mechanism consists of β -CD that can interact via guest-host interaction and GO can interact with phenol via π - π interactions. It has the potential to be used as an adsorbent for naphthols because of its low cost and environmental friendliness (Heydari et al. 2018).

Another study employing rGO/epichlorohydrin cross-linked β -CD (Rout and Jena 2022) shows the adsorption of three phenolic compounds (phenol, 2-chlorophenol, and 2,4-dichlorophenol) from aqueous solutions. The maximum uptake for phenol, 2-chlorophenol, and 2,4-chlorophenol was 659.475, 674.155, and 702.853 mg g⁻¹, respectively. A removal efficiency of 78% was observed for phenol from the industrial waste within 60 minutes. The reactivity of phenolic compounds with disinfectant chlorine produces chlorophenols. Primary contaminants include chlorophenols such as o-chlorophenol and 2,4-dichlorophenol antioxidants. Paper, petrochemicals, dyes, wood preservatives, plastics, insecticides, and trash disposal contain these contaminants. Furthermore, it is created as a side product of chlorine bleaching of pulp and chlorination treatment of potable water. The presence of chlorine groups in o-chlorophenol and 2,4-dichlorophenol makes it extremely harmful in the environment, even at a low dosage (Rout and Jena 2022). The adsorption mechanism is mainly controlled by H-bonding between adsorbent-adsorbate and electrostatic interactions because of charged surface of nanocomposite and π - π interaction between π electron cloud of phenolic ring and oxygen-containing functionality on rGO/ β -CD nanocomposite. During adsorption, the phenol rings as electron-gaining moiety and the rGO/ β -CD nanocomposite's π electron-dense areas operate as electron-releasing sites. As a result, the dispersive interaction of π cloud electrons controls the adsorption of phenolic chemicals onto the composite (Scheme 2). Furthermore, van der Waals forces and hydrogen bonds generate a guest-host inclusion complex between phenolic chemicals and β -CD. As a consequence, additional phenolic molecules are drawn to the surface of the adsorbent, accelerating the removal of phenols from the aqueous medium. Because the interaction between the composite and phenolic compound takes place over the whole rGO/ β -CD nanocomposite surface, the adsorbed phenolic molecules are dispersed evenly on the adsorbent surface. Two distinct electron acceptor–donor interactions occur during the removal of phenolic contaminants: (i) the engagement of the rGO/ β -CD's electron donor -COOH and -OH groups with the electron-acceptor phenyl ring of the phenolic molecule and (ii) the interaction of the rGO/ β -CD's electron-rich -NH₂ with the aromatic ring of the phenol. During the adsorption process, the interaction between the donor and acceptor of the adsorbent and phenolic compounds is determined by the dipole moment of the different functionality available on the basal plane (Scheme 2) (Rout and Jena 2022).



Scheme 2 Schematic diagram for proposed phenolic compound removal mechanism. (Reprinted by permission from [Rout and Jena 2022]; Springer; Environment science and Pollution Research; Synthesis of novel epichlorohydrin cross-linked β -cyclodextrin functionalized with reduced graphene oxide composite adsorbent for treatment of phenolic wastewater; Rout, D.R., and Jena, H.M. copyright [2022])

The removal efficiency of phenol can be improved by using collagen-based double-network composite material. Collagen (extracellular proteins) is a biopolymer that has been found to be significantly preserved among organisms. It is the most common extracellular component of most tissues in mammals, accounting for one-third of all protein found within tissues. Collagen-(2-acrylamido-2-methylpropane sulfonic acid- methacrylic acid/acrylamide)- $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ magnetic double-network nanocomposite hydrogel synthesized via in situ ultrasound-assisted approach can remove 77% phenol and 83% p-nitrophenol from the wastewater. The maximum adsorption capacity of double-network nanocomposite hydrogel was

16.527 mg g⁻¹ and 19.201 mg g⁻¹ for phenol and p-nitrophenol, respectively. The proportion of phenol removal percentage rose as the pH grew from 2–9. Adsorption, in reality, is primarily governed by the ion exchange process. In acidic pH, the removal efficiency is minimally attributed to very weak ionization (or perhaps the absence of ionization) and the development of strong H-bonds throughout the chains. Furthermore, at acidic pH, the adsorbent surface is often protected by “H⁺” ions, preventing adsorbent-adsorbate contact. The adsorbent surface is negative in alkaline conditions due to a decrease in the proportion of H⁺ ions and an increase in the number of OH⁻ ions. As a result of the repulsion of -OH ions as well as adsorbent anionic functions, the greatest % removal efficiency was obtained at higher pH (below 11). At pH 11 or higher, deprotonation of phenol led to the formation of phenoxide anion which results in repulsion between phenol and adsorbent leading to decreased adsorption (Nakhjiri et al. 2021). The presence of nitro (-NO₂) at the para position of phenol leads to increased H-bonding interactions between active functional groups of double-network nanocomposite and hence the removal efficiency of adsorbent p-nitrophenol is higher than phenol.

Chitosans' maximum adsorption capacity for phenol (61.69 mg g⁻¹) can be improved to 89.96 mg g⁻¹ with the addition of MWCNTs to form covalently linked chitosan/MWCNTs nanocomposite (Guo et al. 2019). Further, the use of chitosan/mesoporous silica nanocomposite can remove phenol from aqua solution with a maximum adsorption capacity of 149.25 mg g⁻¹, higher than that of collagen, β-CD, and sodium alginate-based nanocomposites because of its mesoporous structure (Fathy et al. 2020; Feng et al. 2017; Heydari et al. 2018; Nakhjiri et al. 2021). Silica (MCM-48) is a well-known mesoporous silica product with an interlaced and branching porous structure that is ideal for entrapping phenol from water solution due to its consistent porous structure, vast specific surface area, and customizable pore diameter (Fathy et al. 2020). The reusability study suggests a sharp decline in removal percent of phenol on chitosan/mesoporous silica nanocomposite from 92.94–48.13% throughout six reuse cycles owing to adsorbent mass loss. Nevertheless, the adsorbent can still be used up to seven cycles (Fathy et al. 2020).

Conclusions and Future Outlooks

To conclude, water scarcity has become a global concern because of the increasing global human population. This is a high time to restore the contaminant water and store it for the future era. The eco-friendly, cost-effective, and sustainable way is to use biopolymer-based compositions for wastewater treatment. Biodegradability and biocompatibility of the natural polymers can be modified into other better performing composite materials. The tertiary treatment of water using the adsorption approach can be utilized for various agro and industrial sectors, freeing up fresh water for potable purposes. Modified biopolymer-based nanocomposites containing chitosan, sodium alginate, cellulose, β-CD, starch, collagen, etc. as a reinforcing material or matrix have the potential to provide cheaper, environment-friendly, sustainable adsorbent with enhanced removal efficiency. As opposed to their fully

synthetic polymers, biopolymer-based compositions do not accumulate in the environment causing secondary pollution after or during adsorption. Especially use of magnetic biopolymer nanocomposite is a very efficient and less energy-consuming way to retrieve adsorbent after application. Despite good adsorption capacity, compared to hybrid semisynthetic biopolymers, the nanocomposite containing only biopolymer and nanofiller lacks a large specific surface area and high adsorption capacity. The biopolymer-based nanocomposites' adsorption can easily get disturbed by the presence of other contaminants than the target molecules, and hence the enhancement of biopolymer nanocomposites for selective adsorption is still yet to be fully developed. The combined techniques for wastewater treatment can pave the way for future water recycling.

Cross-References

- [Biopolymer-Based Nanocomposites](#)
- [Biopolymer Waste Management](#)

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Hema S., Greeshma U. Chandran, Jyothi P. R., and
Sreedha Sambhudevan

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Abstract

Chitin is a biopolymer widely distributed in nature, and it is the second most abundant polysaccharide after cellulose. Due to its biological activities and usefulness in industry, chitosan and its oligosaccharides have recently attracted a lot of interest. Chitosan is a nontoxic biopolymer made by deacetylating chitin. Chitin, chitosan, and its derivatives are often employed in food science, tissue engineering, and wound healing. Easy techniques for making chitin and chitosan nanofibers have recently been established, and research on the biological uses of chitin and chitosan nanofibers is continuing. This chapter aims to analyze the recent advances in biomedical applications of chitin and its derivatives.

Keywords

Bio-polymer · Polysaccharides · Chitin · Chitosan · Application of chitin

H. S. · G. U. Chandran · S. Sambhudevan (✉)

Department of Chemistry, Amrita Vishwa Vidyapeetham, Amritapuri, Kollam, Kerala, India
e-mail: sreedha@am.amrita.edu

J. P. R.

Department of Chemistry, M A College, Ernakulam, Kerala, India
e-mail: jyothi@macollege.in

Introduction

Chitin is a biopolymer widely distributed in nature, and it is the second most abundant polysaccharide after cellulose. The use of chitin emerged as a suitable alternative for the synthetic polymers, which ruled the society after the World War 11. Chitin occurs in nature as crystalline microfibrils, and due to its crystalline nature, it is found to be less soluble in most of the solvents. This low solubility often restricts the use of chitin in various applications (Roy et al. 2017). The deacetylated form of chitin, chitosan, is found to be soluble in many solvents including acids. Chitin is a polysaccharide consisting of a higher percentage of the acetylated form, while chitosan contains a higher degree of the deacetylated form. Chitosan can be prepared from chitin by (i) partial deacetylation in the presence of concentrated NaOH or (ii) enzymatic hydrolysis using the enzyme chitin deacetylase. Although the main commercial source of chitin comes from crabs and shrimps, it is also found in insects, the cell walls of nematodes, fungi, and yeast (Rouhani Shirvan et al. 2019). In addition to their easy availability, chitin and chitosan are biodegradable, biocompatible, antimicrobial, and are found to be less toxic. Additionally, both of these are in the list of environmentally degradable plastics (EDP). It is due to these advantageous properties, particularly its abundance, that makes chitin and its derivative a suitable polymer for various applications such as biomedical, food and cosmetics, pharmaceuticals, and tissue engineering (Kaczmarek et al. 2019). Chitin is made up of poly β -(1 \rightarrow 4)-*N*-acetyl-D-glucosamine units linked by glycosidic linkages. Abundant hydroxyl, amino, and acetyl groups in chitin and chitosan will provide a tendency for inter- and intramolecular hydrogen bonding, which may increase the crystallinity of these polymers (Fig. 1).

Chitin occurs in different crystalline polymorphic forms such as α , β , and γ . The outer skeleton of crab and shrimp consists of the α -chitin, which is the most dominant form, while β form is seen in the proteins in squid pen and γ in the cocoon of moth. α -chitin has an antiparallel arrangement of individual chains,

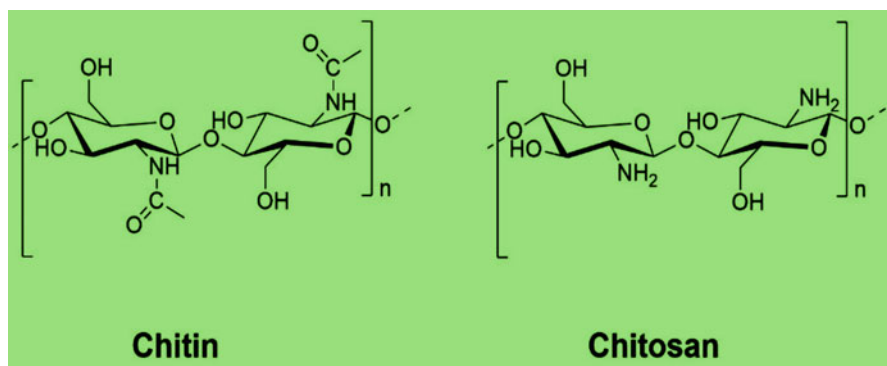


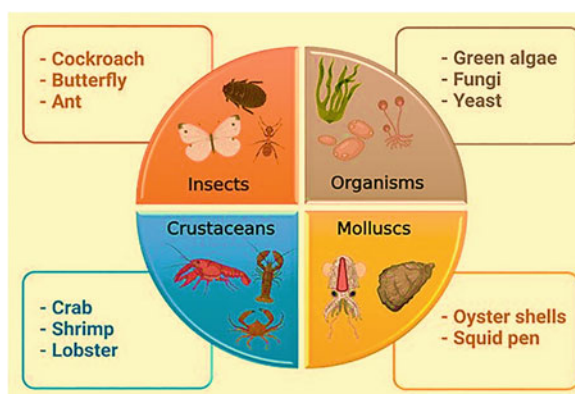
Fig. 1 Structure of chitin and chitosan

which makes it possible for maximum hydrogen bonding between the chains and results in high crystallinity. β -chitin has a parallel alignment of units and a larger distance between the molecules, which makes it more flexible and soluble in solvents (Tamura and Furuike 2014). In γ chitin, we can see a combination of α and β forms. Due to the strong intermolecular interactional forces existing between the molecules in chitin, it is very difficult to utilize chitin for various modifications using organic solvents. For this reason, chitin is often converted to its different derivative forms such as chitosan, carboxymethyl chitin, fluorinated chitin, (diethylamino) ethyl chitin, phosphoryl chitin, mercapto chitin, and chitin carbamates. These chemically modified forms are used in various fields such as cosmetic, food, and textile industries, water treatment, plant protection, and pharmaceutical fields. Chitin and its derivatives have important applications in biomedicine. This chapter is intended to summarize the various biomedical applications of chitin and chitosan (Li et al. 2020; Daraghmeah et al. 2011).

Sources of Chitin

After cellulose, chitin is the polysaccharide that occurs in nature in the greatest abundance. It is extensively dispersed across the animal and plant kingdoms and is a significant renewable resource (Fig. 2). One of the primary sources of chitin and chitosan is an invertebrate group known as the crustaceans, which include crabs, lobsters, and shrimps; it is also a part of the exoskeletons of insects and mollusks, and it occurs in combination with other substances such as lipids, calcium carbonate, proteins, and pigments (Tamura and Furuike 2014; Daraghmeah et al. 2011). Every year, roughly 6.1 million tonnes of crustaceans are harvested from interior and coastal waterways with the goal of producing chitin and chitosan. In addition to this, chitin accounts for around 40% of the remaining wastes created by marine food,

Fig. 2 Different sources of Chitin

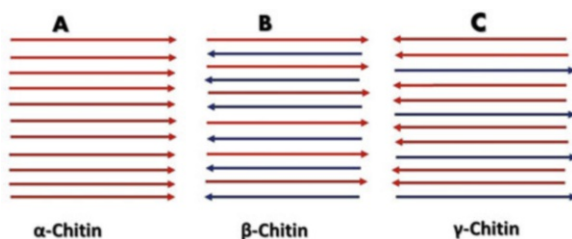


the majority of which are environmental pollutants. A few types of fungus naturally contain chitin, and these fungal biopolymers offer a number of advantages over those extracted from processed seafood. Not all fungus species' cell walls contain chitin and chitosan (Rinaudo 2006). It is known that the fungi belonging to the classifications Basidiomycetes, Ascomycetes, Zygomycetes, and Deuteromycetes have chitin and chitosan in their cell walls. Before stepping up production, it is important to optimize the quantity and characteristics of fungal biopolymers, as they are influenced by a variety of parameters.

In nature, chitin is found in three different crystalline polymorphic forms. They are α -chitin, β -chitin, and γ -chitin. Different orientations of the microfibrils exist within each form; antiparallel chains that make up the α -form, which is widely present and responsible for the polymer backbone's rigidity (strong hydrogen bonding, two per unit cell), are found in the prawn, crab, and lobster shells, as well as beetle shells and fungal cell walls, which are examples of crustacean shells (Shukla et al. 2013; Lizardi-Mendoza et al. 2016). However, β -chitin is composed of parallel chains that create monocyclic crystals with both intramolecular (hydrogen bonds, one per unit cell) and intermolecular interactions. A somewhat more complex arrangement of the chitin chains and crystalline structure may be seen in γ -chitin, which consists of three chitin chains and alternately aligned parallel and antiparallel polymer chains. The presence of γ -chitin is less frequent than that of α - and ∞ -chitin, and it has only been suggested in a small number of studies that beetle cocoon fibers contain it (Velásquez and Pirela 2016) (Fig. 3).

For example, the fungal, insect cocoons, and yeast includes a variety of parallel and antiparallel chains (α -chitin and β -chitin), and the squid pens, pogonophoran tubes, and diatom spines contains only β -form of chitin. The chitinous materials extracted from crustaceans, invertebrates, and arthropods, especially those with hard outer shells, are often shaped like an α -form. This is because the chains are antiparallel, making the arrangement comparatively compact as a result of stronger and more stable hydrogen bonding. In insect cocoons, which feature two parallel threads and one antiparallel string, chitin with the γ -form is most common. The α -form can be converted into the β -form, but not the other way around. Chitin separated from different sources has different crystallization, purity, and polymer chain compositions depending on the source and the amount of chitin that is naturally present there.

Fig. 3 Three different polymorphic forms of chitin



Extraction of Chitin

There are always new sources of crustacean chitin being found after decades of discovery. Now a number of ways to acquire chitin are available in the literature, including chemical, biological, and even a mix of the two to produce an even more efficient procedure. Whatever method is used, the objective is the same: remove all proteins, minerals, lipids, and colors until just the desired substance is left. Due to its efficiency and usefulness, the chemical approach is currently the most used method in industrial production; however, other methods of generating chitin are generally accessible and extremely useful (Hou et al. 2021).

Strong acids and bases are used in the chitin extraction process, which has negative effects on the process such as high material costs, the creation of chemical waste, and low purity levels in the finished product. Biological processes are becoming more and more appealing since they have cheap production costs, do not create high-risk pollutants (unlike chemical processes), and produce high-quality final goods (Younes and Rinaudo 2015). The goal of every procedure described in the literature is to get chitin by removing the minerals and proteins from the source material. In addition to having significant biotechnological utility, chitin produces by-products (like chitosan) that are equally valuable and even more pertinent (Fig. 4).

Fishing sector waste, mostly the shell of prawns, crabs, and lobsters, is the primary industrial source utilized to extract the biopolymer. To eliminate any organic matter adhered to their surface, the crustacean shells are crushed to the proper size (often a few mm) and thoroughly cleansed. In the deprotonation stage, the washed sample was treated with a diluted NaOH solution at an elevated temperature of 60–100 °C for around 30 min. Depending on the treatment, the reaction might

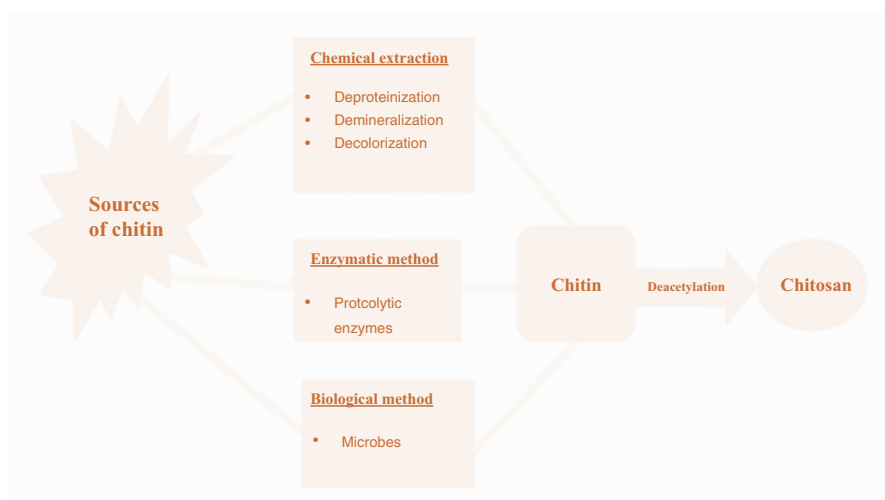
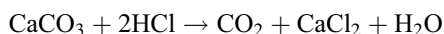


Fig. 4 Standard protocols for chitin and chitosan extraction

continue for up to 4 days. KOH, K₂CO₃, Na₂CO₃, NaHCO₃, Ca(OH)₂, Na₃PO₄, Na₂S, Na₂SO₃, and NaHSO₃ were also used as reagent for the above process of deprotonation. After this stage, the pH of the solution is adjusted to the isoelectronic point to obtain precipitate (Percot et al. 2003; el Knidri et al. 2018). The protein obtained can be utilized as an additive for cattle livestock. The deproteinization methods involving enzyme extracts or isolated enzymes and biological fermentations have shown some success, since they reduce the chemical breakdown of chitin and provide cleaner operations. The problem with enzymatic/microbiological treatments is that they take time and leave the material with 1–7% of residual protein.

In the demineralization stage, the calcium carbonate present in the shells can be eliminated via treating with dilute hydrochloric acid. The stoichiometric ratio of acid has a crucial role in the demineralization stage so as to control the depolymerization and deacetylation of chitin. The process follows the below equation:



To minimize thermal degradation, high temperature treatments must also be avoided. Use of the complexing agent EDTA (ethylenediamine tetra acetic acid) in basic solutions is an alternate treatment for demineralization (Acosta et al. 1993).

The staining is a reliable sign of the material's contaminants. This is achieved through the depigmentation process. The presence of pigments such as astaxanthin, canthaxanthin, astacene, lutein, and -carotene is primarily responsible for the color of crab shells. These pigments are often extracted at room temperature with acetone, chloroform, ether, ethanol, ethyl acetate, or a combination of solvents (Percot et al. 2003; el Knidri et al. 2018).

Application of Chitin and Its Derivatives

Pharmaceutical and Biomedical Application of Chitin and Chitosan

Chitin and its derivatives are excellent biomaterials with a wide range of applications despite all of their distinctive qualities. Because of its biocompatibility, biodegradability, and low toxicity, chitosan is a particularly beneficial polymer for biomedical applications. Chitin is also known to be biocompatible and biodegradable, and it has been used for a variety of things including suture threads, bandages for treating wounds, and sponges. However, because of its insolubility in water and mild reactivity, it has often gotten less attention than chitosan. With its hierarchical structure, multifunctionality, excellent light management, and exceptional mechanical capabilities, chitin offers a wealth of opportunities for us to employ it as a cutting-edge material (Azuma et al. 2014).

In Drug Delivery

Chemical modifications possible in chitin and chitosan, together with their additional qualities like easy bioadhesion, crosslink formation with other polymers, different

kinds of gel formation, and antimicrobial activity, enable researchers in using these polysaccharides in drug delivery. Chitin nanofibrils were integrated with alginate-based microspheres by Lin et al., and they found an increase in storage modulus and loss modulus which confirms the fibril reinforcing function (Parhi 2020). Thus, prepared composite microspheres showed a smooth surface morphology, suggesting an enhancement in structural integrity by the addition of nanofibrils. The encapsulation efficiency of these composite microspheres was tested using theophylline, and it was found that more than 55% encapsulation was achieved by the 3D network of the nanofibrils, which helped in the controlled drug release. Chitosan and alginate-based porous composite hydrogels mixed with bioceramic powders as a dispersed phase were developed and used in drug delivery and tissue engineering. Carboxymethyl chitin (CMC) nanoparticles were used as potential cancer drug delivery systems. A hydrophobic cancer drug, 5-fluorouracil (5-fu), was loaded into the CMC nanoparticles and the drug release studies showed a sustained release at the pH 6.8. Additionally, the prepared nanoparticles showed antibacterial and ferromagnetic behavior, which makes them useful in drug tracking system. O-carboxymethyl chitosan (O-CMCS) hydrogel beads were prepared with several modifications, and their pH-sensitive characteristics were investigated. The drug entrapment efficiency was tested and was found that chitosan-coated calcium-O-CMCS hydrogel beads showed higher entrapment efficiency and proper protein release properties. Thus, it was concluded that these hydrogel beads form promising protein drug carrier for the site-specific release in the intestine. Chitosan/carboxymethyl cellulose polyelectrolyte complexes were synthesized by Luppi et al. as vaginal inserts for the local delivery of chlorhexidine digluconate. O-CMCS microspheres were also used as a potential drug carrier (Das et al. 2022; Rivas et al. 2022). Several other modified CMC derivatives also presents promising results in the field of drug delivery. Carboxymethyl chitin microspheres (CMCH-Ms) were prepared through cross-linking in an aqueous two-phase system by increasing temperature without using organic solvents, surfactants, or cross-linking agents. Naproxen and ropivacaine hydrochloride were used as model drugs for drug loading by using solid-in-water (S/W) immersion in aqueous system. The CMCH microspheres and the drug loading microspheres were embedded in the hydroxypropyl chitin (HPCH) hydrogels to form CMCH-Ms/HPCH gel composite scaffolds. These scaffolds showed good biodegradability and biocompatibility and less burst drug release with a sustained drug release.

Drug release kinetics of some model drug systems including, curcumin, progesterone, and mesalamine, for 24 h in physiological conditions were investigated in pectin-chitosan hydrogels as drug carriers. Chitosan film was developed using pectin or hydroxypropylmethyl cellulose, and a model drug, miconazole nitrate, was loaded into the films. These films of chitosan-hydroxypropylmethyl cellulose were found to be the most suitable as drug carriers. Chitin nanocrystals were synthesized and cross-linked with glutaraldehyde, and these cross-linked crystal were used as scaffolds. Curcumin was used as the model drug, and the drug release profile showed promising results. Cytotoxicity studies were also done, which affirm the potential of the synthesized nanocrystals in drug delivery and tissue engineering. Chitosan films

were prepared with varying molecular weight and acetylation degree chitosan-depolymerization-products and were applied as drug delivery materials. It was found that by varying the molecular weight and acetylation degree of chitosan depolymerization products, several properties like water resistance, mechanical, and antibacterial properties were changed to a considerable extent. In vitro ciprofloxacin (CFX)-release behavior was also studied on these films after cross-linking using glutaraldehyde. These cross-linked CFX-loaded films showed increased water resistance, optical, tensile strength, and thermal properties to the unloaded films. A thermosensitive chitosan-based hydrogel was prepared for the sustained release of latanoprost, which is used to control ocular hypertension. This was found to be biocompatible with a sustained drug release and was proposed as an alternative to traditional eye drops for the treatment of glaucoma (Nayak et al. 2022).

In Tissue Engineering

The inadequacy of the engineered tissue structures to endure the mechanical stress that may occur under in vivo settings at the moment of implantation is a challenge in tissue engineering and regenerative medicine research. Additionally, the scarcity of suitable biomaterials as temporary matrix is also a major challenge in this area. However, chitosan, by virtue of its properties like biocompatibility, cell adhesion, differentiation, and proliferation, is used widely in the field of tissue engineering. It finds its application in bone tissue engineering, cartilage regeneration, and nerve and liver tissue engineering (Yang 2011). Nowadays, much attention has been given by the researchers to the development of polymer nanofibers with various diameters ranging from several micrometers to nanometers using electrospinning. Biodegradable and biocompatible hydrogels were derived from chitosan and hyaluronan through metal-free click chemistry, and it was found that these could be used as an injectable scaffold for adipose tissue engineering. On account of their structural similarities with glycosaminoglycans and mechanical and morphological properties with collagen, chitosan derivatives are excellent substitutes in tissue engineering. Chitosan derivatives are effectively used in skin tissue engineering (Jayakumar et al. 2011; Wan and Tai 2013). A bilayered chitosan hydrogel was prepared for the treatment of burns in pig models. It was found that efficient reconstruction of dermal-epidermal junction and dermis as well as the re-epithelialization of wound could be attained. Various chitosan composites with collagen and elastin were extensively used as scaffolds in skin regeneration to increase the tensile strength and flexibility of the prepared scaffolds. A polyelectrolyte gelatin-chitosan hydrogel was prepared for 3D bioprinting in skin tissue engineering (Soroush et al. 2022). Electrospinning was found to be an efficient synthesis of the scaffolds in tissue engineering. Poly (L-lactic acid)/collagen nanofiber that was coated with chitosan was synthesized and tested for sustained release of aloe vera (AV) gel. It was shown that these nanofibers had an enhanced effect on mouse fibroblast attachment, with improved viability and proliferation. The composites of chitosan and hydroxyapatite hydrogels were reported to show a wider scope in bone tissue engineering. HVP functionalized with chitosan was tested for osteoblast proliferation, differentiation, and bioadhesion, and it showed best results compared to the Chit-RGD. Chitosan-collagen composite films have been shown to produce promising

results in osteoblast proliferation, differentiation, and matrix mineralization in MC3T3-E1 cells. Chitosan-gelatin composites were also reported as better scaffolds, and these composites when incorporated with hydroxyapatite, remarkably improved cell attachment and growth on chitosan-gelatin matrices. Many synthetic polymers, such as polylactic acid, poly-methyl methacrylate (PMMA), poly-caprolactone, and polyethylene glycol, are blended with chitosan to show enhanced mechanical properties, and these polymer composites were found to be biocompatible as well (Bi et al. 2021). An in situ-forming hydrogel was prepared from chitosan, gelatin, and bioactive glass. This is an injectable hydrogel and is stimulated by body temperature. This hydrogel can be administered as a fluid using a syringe and a needle, making it a unique thermosensitive, lowly invasive system. Graphene and graphene oxide have been successfully combined with chitosan for applications in bone tissue engineering. These composites reinforce organic-inorganic hybrid scaffolds because of their superb mechanical properties, and due to the presence of free π electrons, they possess antibacterial properties in addition to cell adhesion, proliferation, and differentiation. Chitosan and cellulose nanofiber-based injectable suspensions were developed for intervertebral disc (IVD) repair and its regeneration through the augmentation of one of its regions, the nucleus pulposus. Efficient promising transparent injectable solution was developed for IVD regeneration, which offers an alternate for open surgery (Croisier and Jérôme 2013). This is a biocompatible and nontoxic chitosan-based hydrogel. It shows a constant storage modulus over a wide range of strains, which resembles the IVD ECM structure and makes it a suitable scaffold for cells of IVD and regeneration. Vascular layer-by-layer patches have been developed in which the layered deposition improved attachment and proliferation of cells. Different layers are built up from oppositely charged components. Vascular tissue engineering scaffolds were developed using chitosan-immobilized polyethylene terephthalate and chitosan-PVA films, which showed improved cell adhesion, morphology, and growth. Chitosan-PVA combination showed improved cell attachment while maintaining the physical properties of the scaffold. A blood vessel tube with blended PLLA-chitosan-collagen electrospun fiber was developed by electrospinning, which generated blood vessels with high surface area-to-volume ratios. These prepared blood vessels showed improved cell viability and hemocompatibility, good tensile strength, and good burst pressure. Periodontal disease causes irreversible loss of periodontal tissues and ultimate tooth loss (Liu et al. 2011). Chitosan was combined with different organic and inorganic materials in the biodental field. Incorporation of DNA into a chitosan and collagen matrix was found to regenerate periodontal tissue. This chitosan/collagen matrix was porous which embedded chitosan/plasmid DNA nanoparticles encoding platelet-derived growth factor. Due to its porous structure, the DNA was released in a steady manner for about 6 weeks. Analysis was continued for 2 weeks, and it was revealed that the material was capable of initiating protein at high levels. Moreover, it also developed periodontal connective tissue-like structures. Alginate microspheres were prepared by electrospraying and then incorporated into previously formed chitosan gels. They were tested for controlled delivery of bone morphogenetic protein-6 (BMP-6), which was the major growth factor for promoting the periodontal tissue regeneration (Bi et al. 2021).

In Wound Healing

Since the early 1980s, chitosan and its derivatives have been used to treat skin and wounds. Beschitin, a chitin material, was used for dressing skin and nasal wounds. Faster wound healing was observed with chitin and its derivatives where they improve the hemostasis by the production of collagen in fibroblasts. It also helps in the formation of coagulum, with red blood cells responsible for the blood coagulation (Khor and Lim 2003). *N*-acetylglucosamine, which is an extracellular macromolecule crucial to wound healing, is the monomeric unit of chitin, often found in hyaluronic acid. For uses ranging from straightforward wound dressings to complex artificial skin matrices, chitin can be used since it ought to have properties that encourage quick dermal regeneration and expedited wound healing. Basic fibroblast growth factor (bFGF) infusion enhanced the pace of healing, and hence chitosan was an effective substance for wound healing (Mizuno et al. 2003). A step crucial for rapid wound healing in polymorphonuclear (PMN) cell infiltration at the incision site was facilitated by chitosan in the form of chitosan-cotton, which augmented wound healing. For use in healing process, a composite material made of α -chitin and nanosilver were developed (Ueno et al. 1999). These α -chitin/nanosilver composite materials were found to have potent antibacterial activity against *S. aureus* and *E. coli*, as well as a strong blood clotting capacity (Mathew et al. 2010). It has been suggested that burn dressings be made with electrospun nanofibrous materials based on chitosan. For burns of degrees IIIa and IIIb, chitosan nanofiber mats were produced and tested as wound dressings. The study revealed that chitosan nanofiber dressings effectively sucked up secretions, oxygenated the area, were designed to protect from infections, and initiated the function of skin tissue regeneration. Damage to the wound from mechanical action during removal was averted by the degradation of these materials (Cai et al. 2010). A crucial component for the physiological activities of freshly created tissue is vascularization. In growing tissue, the polysaccharides actively contribute to neovascularization. By implanting chitosan in the cornea, researchers were able to show that it can encourage neovascularization. Chitosan is said not to cause any further inflammatory reactions. According to recent clinical research, modified chitosans that include amino acid moieties and substituents at the N-atom are helpful in the healing of wounds. The development of vascularized, nonrefractive tissues with well-oriented collagen is often induced by these factors. Capsular tissue development is inhibited by modified chitosans. Due to chitosan's angiogenic properties, some cartilaginous tissues may also be healed (Singh and Ray 2000).

In Artificial Kidney Membrane

It has been suggested that chitosan membranes, which have great mechanical strength and urea and creatinine permeability, might be used as prosthetic kidney membranes. Serum proteins are not able to pass through these membranes. In order to give improved selectivity and higher dialysis rates for medium- and large-size molecules like uric acid and the like, it is necessary to improve hemodialysis membranes. To fulfill the needs of dialysis applications, either the modification of preexisting polymeric membranes or the synthesis of novel polymeric membranes

are being developed (Nakatsuka and Andradý 1992). The pH-dependent permeability coefficients of a membrane consisting of chitosan and PVA, cross-linked with different concentrations of glutaraldehyde, riboflavin, and insulin, were identified (order of $106\text{--}107\text{ cm}^2/\text{s}$). Additionally, the impact of chitosan cross-linking and blending with PVA on the permeability of vitamin B-12 was investigated. The partition coefficients for the membrane under study were essentially constant ($K = 0.4$), supporting the idea that vitamin B-12 is transported by “pore-type” processes (Chandy and Sharma 1992). It has been reported on a number of membranes made by mixing chitosan and PVA and immobilizing bioactive molecules like prostaglandin E1 (PGE1) on heparin. These membranes have strong small molecule permeability and significantly reduce platelet attachment (Blair et al. 1987). Chitosan can be altered to have dialysis capabilities via graft copolymerization and blending with water-soluble polymers. Chitosan and polyvinyl acetate (PVA) combine to create a transparent, uniform blend with a tensile strength that is higher than the sum of the component parts, as was proved in a study by Srinivasa et al. In order to study the active transport of the halide ions over the chitosan/PVA membrane, created a cross-linked chitosan/PVA blend were created with a set proportion of the cross-linking agent. In a heavily cross-linked membrane, Reinhart and Peppas investigated the diffusion of bovine serum albumin (Reinhart and Peppas 1984). The transport of alkali metal ions and smaller molecular weight solutes via various polymeric membranes made from chitosan derivatives was proposed in other research. Chitosan and its derivatives were used to create a variety of membranes (Hirano 1978) and the membranes demonstrated better dialysis characteristics. In order to improve blood compatibility and permeability, vinyl monomers were added to chitosan membranes using ^{60}Co γ -ray irradiation (Singh and Ray 1999). According to certain researchers, albumin-blended chitosan membranes had better permeability qualities for smaller molecules than the conventional cellulose membranes and demonstrated the greatest reduction in platelet adherence in comparison to other membranes. The permeability of KCl and sucrose was examined in a glycol-chitosan membrane modified with thiol groups. The study demonstrated that permeation control may be accomplished by adjusting the cross-linking agent’s concentration.

In Cancer Therapy

In the world, cancer accounts for 13% of fatalities, and its prevalence is rising. By 2030, 12 million individuals are predicted to develop cancer, with lung, prostate, colorectal, and breast cancer being the most frequent forms (Shanmuganathan et al. 2019). It is essential to utilize drug carriers that are secure and capable of effectively delivering the medications to the malignant tissue without endangering healthy cells as part of anti-cancer therapy. In chemotherapy, chitosan and chitin have been utilized as drug delivery vehicles. 5-fluorouracil (5-Fu), an anticancer medication, was loaded onto carboxymethyl chitin (CMC) nanoparticles, which are soluble in water and created by cross-linking with CaCl_2 and FeCl_3 (Jayakumar et al. 2010a). It has been demonstrated that chitin and chitosan’s chemical alteration significantly affects their physiological characteristics, biodegradability, and interaction with other molecules (Jayakumar et al. 2010b).

Therefore, in order to make use of chitin and chitosan, it is crucial to identify methods that may increase their solubility in typical solvents. Chitin carboxymethylation has previously been demonstrated to improve chitin's water solubility while maintaining biocompatibility and preventing the development of antibodies (Jimtaisong and Saewan 2014). They are created by a condensation reaction between monochloroacetic acid and chitin powder using isopropyl alcohol as the solvent. In a mouse model, it was found that the medication was delivered in a regulated and sustained manner and that the nanoparticles were not harmful to normal fibroblast cells. CMC nanoparticles have shown antibacterial activity and ferromagnetic qualities that can be employed to distribute medications utilizing tracking devices. Due to its beneficial characteristics, carboxymethyl chitin may also be utilized as a material for wound dressings, but it must be cross-linked to keep its integrity (Jayakumar et al. 2010b). Chitosan polymers have been utilized to deliver medications in the treatment of several cancer types in a number of forms, including hydrogels, nanoparticles, and nanofibers. Chitosan polymers are excellent candidates for chemotherapeutic target therapy because of their diverse characteristics. By altering the surface moieties, other properties can be obtained, such as membrane-penetrating peptides, monoclonal antibodies, or surface receptors that target certain cancer cells. Chitin is extracted from shrimp shells to stabilize silver nanoparticles (AgNP) and treat human hepatocellular carcinoma in an endeavor to develop appropriate carriers for cancer therapy (Vijayakumar et al. 2020). Because they are harmful to human cells, Ag nanoparticles have been employed in the therapy of cancer. Their disadvantage is that they may also harm healthy bodily cells. As a result, using a carrier that can only access cancer cells is necessary when using AgNP to treat cancer. Both chitin and chitosan, which are considered to be safe biological sources, have been demonstrated to have anti-proliferative effects in various human malignancies, including colon and lung cancers, two of the most prevalent cancers in the world (Kurishima et al. 2017). Applications for tumor-targeted medication delivery using nanoparticles made of chitosan are shown in Fig. 5.

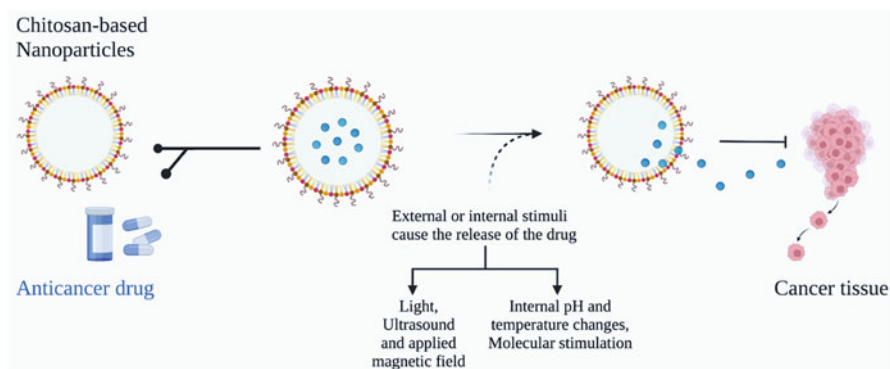


Fig. 5 Applications of chitosan-based nanoparticles in the delivery of drugs to tumors

In Artificial Skin

Chitosan that resembles glycosaminoglycans structurally may be used in the creation of artificial skin. A nonantigenic membrane that serves as a biodegradable template for neodermal tissue was designed for artificial skin that is suitable for long-term chronic usage (Dagalakis et al. 1980). A material for covering wounds has been created using polyelectrolyte complexes of chitosan and sulfonated chitosan. Oligomers of chitosan that have been broken down by tissue enzymes have been found to speed up wound healing and to be effective in regenerating skin tissue around wounds. An artificial skin made of chitosan-collagen composite is produced by a pharmaceutical company called Katakurachikarin in Hokkaido, Japan, and appears to speed up the healing of surgical wounds or burns. Chitosan and gelatin were combined to create a novel absorbable framework for artificial skin that was explored using freezing and lyophilizing techniques. The chitosan-gelatin cross-linked scaffolds were made, and an artificial bilayer skin was created by co-culturing fibroblasts and keratinocytes. The artificial skin created was elastic and had good mechanical characteristics. Additionally, it was discovered that when implanted under the skin of rabbits, it did not cause any unfavorable inflammatory reactions. The *in vitro* cell culture test revealed no contraction, and the scaffolds were biocompatible and biodegradable after implantation (Islam et al. 2017).

In Stem Cell Technology

Embryonic stem cells, post-natural or adult stem cells, and induced pluripotent stem cells are the three main categories of stem cells (Wu and Hochedlinger 2011). Biological materials, such as chitin and its compounds, can assist in realizing stem cells' enormous potential in regenerative medicine using cells. Chitin was used for the *in vitro* growth of programmable stem cells (Lu et al. 2012). The first chitin that was water soluble utilized to create an interfacial combination with alginate acid that encapsulates cells in fibers. There are two ways to spool these fibers: around a flat surface or tangled to create structures for the long-term culturing of cells, which is unique to water-soluble chitin and suited for encapsulating cells. Complex polyelectrolyte chitin and alginate fibers assisted the process of self-renewal. Comprising two induced pluripotent stem cells and two embryonic stem cells' lines for at least ten *in vitro* passages, whereas cells kept their karyotype constant. This use of chitin is due to the creation of a 3D hydrogel environment in which the stem cells multiplied into aggregates without adhering to the cells.

In Ophthalmology

Chitosan, a polycationic biopolymer made from chitin that was alkaline deacetylated, was selected as the carrier for ophthalmic formulations because it possesses a number of beneficial biological characteristics, including biodegradability, nontoxicity, and biocompatibility. It has become necessary to develop pharmaceutical formulations that provide sustained release, increased bioavailability, and decreased frequency of administration due to the occurrence of diseases like glaucoma, age-related macular degeneration, diabetic macular edema, diabetic

retinopathy, or dry eye syndrome, which require drug delivery for a prolonged period of time. Overcoming ocular obstacles without inflicting lasting tissue damage is a major hurdle in reaching this aim. Chitosan became commercially available in 1990, and various studies were conducted to explore its potential as a pharmaceutical excipient. It is a good choice for ocular formulations because of its mucoadhesiveness, biodegradability, biocompatibility, and nontoxic nature. Chitosan solutions do not disrupt the precorneal tear film due to their pseudoplastic and visco-electric qualities.

For the treatment of glaucoma, ocular inserts made of chitosan have been developed as an alternative to those that release brimonidine tartrate. Analytical characterization of inserts was carried out utilizing FTIR, SEM, and DSC. Additionally investigated on Muller cells were swelling capacity, active substrate release profile, and in vitro bioavailability. Brimonidine tartrate was physically diffused across the polymer chains, according to the study's findings. For 30 days, the inserts release the active ingredient without causing any harm. They furthermore have the benefit of not containing preservatives (de Souza et al. 2016). Chitosan formulations with a prolonged precorneal residence period were tried because of its mucoadhesive properties as well as its capacity to improve solution viscosity properties. In fact, an ionic interaction with the negative charges of the sialic acid residues in the mucus has been hypothesized as a mechanism of mucoadhesion due to its positive charges at neutral pH. For longer precorneal medication residence durations, the mucoadhesive polysaccharide chitosan was examined as a possible ingredient in ophthalmic gels. This cationic carrier was predicted to slow down medication excretion through the lacryme by increasing the viscosity of the solution and reacting with the mucus' negative charges (Felt 1999).

Due to their biocompatibility and transparency, hydrogels are typically used to make soft contact lenses. When using contact lenses, hydration is necessary because it helps oxygen reach the cornea. It is advised to wear contact lenses together with eye drops since dehydration causes dry eye syndrome. Dexamethasone might be made more readily available to the eye by being mixed with chitosan nanoparticles and then imprinted on pHEMA hydrogel contact lenses, according to a study by Behl et al. In vitro release experiments were also researched. The lens was clear, with an average transmittance of 95–98%, and the dexamethasone release rate was 55.75 percent in 22 days. The study found that within the first 10 days, dexamethasone had a 72% higher bioavailability than eye drops. According to the study's findings, using contact lenses containing dexamethasone-infused chitosan nanoparticles has beneficial therapeutic effects (Tashakori-Sabzevar and Mohajeri 2015; Behl et al. 2016). When chitosan is used in ophthalmic delivery systems like nanoparticles, nanomicelles, nanosuspensions, liposomes, microemulsions, microspheres, in situ gels, inserts, contact lenses, or mini-tablets, the active ingredient is retained for longer at the eye level while also having its bioavailability increased. As a result, the dosage will need to be given less frequently, and the patient will be more compliant, which will enhance his quality of life. These chitosan-based products do not harm tissues, change ocular barriers permanently, or obstruct tear production (Popa et al. 2018).

Other Miscellaneous Applications

In Wastewater Treatment

Currently, the primary commercial use of chitosan is in the treatment of industrial effluent. As chitosan has a limited beneficial effect, It is simpler to remove metal ions from waste streams or contaminated areas when an agent has a positive charge and binds to metal ions. The commercial chitosan from shrimp and crab waste shell and synthetic polyelectrolyte used in turbidity reduction were shown to be equal to or superior in terms of usage for recovering organic compounds in wastewater. The physical chemistry and mechanistic properties of chitin and chitosan, which encompass the electrostatic interactional behavior (Nechita 2017) of functional groups, clearly define their potential as scavengers for the removal of target metal ions in contaminated water.

Chitosan appears to be more efficient than other polymers in these particular uses, including synthetic resins, activated charcoal, and even chitin itself. Additionally, the amino group in chitosan is a powerful functional group that may be chemically changed to produce various chitinous derivatives with particular advantageous properties as powerful absorptive agents. In addition to the crucial role played by electrostatic interactions, the high hydrophilicity of chitosan owing to hydroxyl groups and primary amino groups on the surface is accounted for by the noticeable biosorption behavior of chitosan for heavy metal removal (Hubert Ribeiro and dos Santos 2019). Additionally, chitosan contains a flexible polymer chain in that may be configured in a way that facilitates the biosorption phenomena.

Cu (II) ion-containing wastewater was treated using sodium alginate and chitosan, according to research by Qin et al. (0975–0991 (Online); 0971-457X (Print)). They looked at how various factors affected this ion removal (Qin et al. 2007). According to the findings, chitosan and sodium alginate worked better together than they did separately. Hexavalent chromium (Cr (VI)) removal from wastewater using FeO-nanoparticles-chitosan composite beads (CSNZVI beads) was examined by Liu et al. The thermodynamics investigation revealed that the endothermic and spontaneous removal of Cr (VI) by CS-NZVI beads was due to the sorbent's high affinity for Cr (VI) ions and the increase in randomness at the solid-solution interface during the adsorption process (Liu et al. 2012). In the successful application, this finding will be extremely helpful to comprehend the impacts of NZVI on heavy metal Cr (VI) removal from wastewater. Pesticide removal from wastewater was explored by Dwivedi et al. (Ueno et al. 1999) using hydrogel beads made of chitosan and gold nanoparticles. The collected findings demonstrated that the synthetic sorbent had high pesticide removal capacity (Dwivedi et al. 2014). The efficacy of chitosan as waste treatment agents varies depending on their physical properties, such as molecular weight, crystallinity, deacetylation, particle size, and hydrophilicity. The scientific community is quite interested in determining which biosorbent is best for eliminating pollutants, though. Consequently, well-organized research is being done in a variety of fields throughout the world (Sarode et al. 2019).

Agricultural Applications

Biocontrol employing natural substances like chitin, a carbohydrate chain polymer, and its derivatives is a viable method as the need for sustainable agriculture grows more urgent. Plants' built-in defense systems are induced or strengthened by chitin and its derivatives. They are acknowledged as growth regulators, growth promoters, and inducers of secondary metabolite formation in plants. As fertilizers, soil conditioners, plant disease preventatives, antitranspirants, ripening inhibitors, and seed and fruit coatings, they have positive benefits. Chitin and chitosan are powerful inhibitors of bacterial and fungal plant disease development, which causes higher plants to mount defense responses (Shamshina et al. 2019).

The majority of synthetic plant protection chemicals are poisonous, bioaccumulative, persistent in the environment, and dangerous to both people and animals. Recently, there has been a lot of interest in using chitin polymer as an environmentally acceptable natural alternative to synthetic herbicides to manage plant diseases and control plant growth. Chitin is a substance that is used in agriculture to make fertilizers and is regarded as a "nitrogen type" "organic" fertilizer. A C:N ratio of around 25–30 is maintained by effective fertilizers. More than a 25:1 carbon-to-nitrogen ratio with excess carbon often indicates that fertilizer will breakdown slowly. Contrarily, if there is a surplus of nitrogen and the C:N ratio is too low, this might result in the soil losing its ability to hold onto plant nutrients. For completely deacetylated chitosan, the C:N ratio of chitin and chitosan ranges from around 6 to 7 for chitin. The amount of nitrogen (N), phosphorus (P), and potassium (K) that the product contains is another crucial ratio; this is typically denoted on fertilizer labels as the "N-P-K ratio"; chitin contains neither phosphorus nor potassium (Shamshina et al. 2020). According to the mode of action, chitin or chitosan has a fertilizer impact by either biodegrading into ammonia-derived compounds in the soil, which have a fertilizer effect on their own owing to the presence of amino groups, or by encouraging the development of certain microbes.

Any poison that kills a living thing is considered to be a biocide, which includes fungicides that are active against fungi, oomycetocides that are active against oomycetes, bactericides that are active against bacteria, antivirals that are active against viruses, nematocides that are active against nematodes, and insecticides that are active against insects (Malerba and Cerana 2019). Since chitosan is an antibacterial agent on its own, chitosan derivatives showed strong "direct" antifungal activity; however, chitin does not have much in the way of "direct" antibacterial activities since it lacks free amino groups. According to research, chitosan's "direct" antibacterial action is directly related to DA, or the quantity of free amino groups. Chitosan's antibacterial activities increased with the amount of protonated amino groups present, whereas a low DA was associated with the absence of such characteristics (Sharp 2013).

Chitin naturally serves as scaffold material in insects; hence, it is not anticipated to have pesticide capabilities. But chitin derivatives work as antivirals, or something that can kill a virus. According to reports, chitosan can boost a plant's immune system and prevent the growth of viruses. Chitosan's ability to inactivate viruses is not explained, and there has been no research on how underivatized chitin affects

viruses. According to one theory, chitosan's efficacy against plant viruses is caused by altering the plants' reaction to infection and preventing the spread of viral particles (Pospieszny et al. 1991). Despite the fact that chitin lacks "direct" antibacterial action, it is frequently employed as a fungicide because of the polymer's eliciting activity and indirect suppression of the pathogens brought on by chitin breakdown by-products. Chitin's fungicidal efficacy is influenced by the molecular mass of the substance. Furthermore, soil fumigation, irrigation, or the addition of chitin-containing mixes to the soil are other ways that chitin is used as a plant nematicide. Chitin has been demonstrated to provide a physical barrier that stops pathogens from entering healthy plant tissues and enhances the lignification of injured leaves. It is a solitary seed treatment that is applicable. Chitin is used as a postharvest biocontrol treatment and to strengthen the resistance of fruits to disease in food postharvest applications (Shamshina et al. 2020; Malerba and Cerana 2019; Milholland 1973).

In Food Industry

It is well recognized that chitin and its derivatives offer a wide range of biological activities, such as antioxidant and antibacterial effects, as well as many other qualities that might be employed in the food market to enhance food quality, safety, and shelf life. The food sector can employ chitin and its derivatives as food preservatives (Sayed-Elahl et al. 2019). They can shield food from microbial degradation owing to their antibacterial activities. The positive charges of chitinous materials have been shown to interact with the negative charges of bacterial cell walls, causing leakage of the internal molecules of microorganisms. This interaction has been connected to the antibacterial activity of chitinous materials. Chitosan is a substance that is added to food to prevent the growth of bacteria, hence preventing bad looks, off tastes, and financial losses. El-Diasty, Nesreen, and Hoda (2012) discovered that cheese's mycological quality was enhanced by the addition of chitosan. Shelf life was increased, and mold and yeast development were prevented (Hamed et al. 2016).

Chitosan's antibacterial qualities have also been applied to active edible packaging. Chitosan has been used to create biofilms that enable long-term food storage. Vegetables, fruits, cereals, and fish that have an antimicrobial coating prevent microbial invasion because chitosan serves as a protective barrier to improve the food's flavor and nutritional value. Edible biopolymer films can be employed as carriers of bioactive substances to improve food quality in addition to serving as a protective barrier. Dietary fiber has been obtained from chitosan and its low-molecular-weight derivative. They are regarded as functional foods because digestive enzymes cannot break them down, allowing them to act as prebiotics. They encourage the growth of advantageous microorganisms in the digestive system. They can also serve as stabilizers and thickeners (Manigandan et al. 2018).

In Textile Industry

Chitosan has been used in the textile industry as an eco-friendly finishing agent for more efficient materials. Textiles provide attached bacteria food and a place to live,

which can damage human health and spread illness. Antibacterial treatment of textiles is crucial to preventing this (Qamar et al. 2020). *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* are among the microorganisms that chitosan exhibits broad-spectrum antibacterial action against. Chitosan is primarily used in two methods to create antibacterial materials. The first method involves applying chitosan to the fabric's finish. In order to graft chitosan onto cotton textiles under UV irradiation, Aloson et al. employed citric acid as a cross-linking agent and sodium dihydrogen phosphate as a catalyst. This provided the textiles with good antibacterial capabilities (Moattari et al. 2018; Lee et al. 2017).

Textile items are less attractive and comfortable because of their tendency to wrinkle and undergo permanent deformation due to their relatively poor elastic properties. Fiber alteration can enhance the fabric's anti-wrinkle efficacy. A simple and efficient method to raise the fabric's anti-wrinkle effectiveness is to finish with chitosan. Typically, chitosan is first dissolved in 1% w/v acetic acid to create a chitosan solution. The cloth is then completed in the chitosan solution using a standard dipping-rolling-baking method. After drying, the water-insoluble protective layer created by the chitosan solution forms on the fabric's surface, strengthening and lessening the fabric's ability to distort. Additionally, chitosan macromolecules fill in the fiber micropores, where a significant number of intermolecular hydrogen bonds are generated between the hydroxyl and amino groups of chitosan and the polar residues of the fiber molecules. This makes the amorphous areas of the fibers stronger and less mobile, giving the fabric wrinkle resistance (Xiao 2018).

Chitosan was initially utilized in the textile industry as a dye-deepening agent. Chitosan is regarded as the best fixing agent for anionic dyes since it is a cationic polymer. Chitosan may be used to color without adding salt, along with a few other additions. In the textile business, antistatic fabric finishing is growing in popularity. The chitosan-finished fibers have an ion-conducting activity in water and may swiftly leak the static charges produced by friction because NH_3^+ is present in the molecular structure of chitosan (Ifuku 2014). When the charge on the chitosan molecule is in opposition to the surface charge on the fiber, electrical neutralization is created. Additionally, the chitosan molecules are extremely hygroscopic and produce a continuous water layer on the surface of the fiber due to the presence of several strong polar groups, such as hydroxyl groups and amino groups. Chitosan may be mixed with traditional textile fibers like wool, cotton, silk, and polyester with ease and with strong interactions thanks to its structural features. Chitosan can be used as a finishing agent or fiber modification to create functional textiles with antibacterial, anti-wrinkle, coloring, and antistatic qualities. The use of chitosan in the textile industry not only enhances the functionality and value of textiles but also encourages the effective utilization of natural resources (Vellingiri et al. 2013; Zhou et al. 2019).

In Cosmetic and Dermatological Applications

Chitosan is frequently used in cosmetic and skin care products because it supports the extracellular matrix, treats acne, maintains skin hydration, tones skin, and enhances the skin's natural barrier function. Chitosan is a natural stimulant

that promotes good histoarchitectural tissue organization and optimal collagen structure throughout the skin regeneration and wound healing processes. This makes it a perfect component for antiaging skincare products and wound healing (Aranaz et al. 2018). Chitosan has excellent film-forming capabilities and a bioadhesive nature. Therefore, it can serve as the active component or act as a delivery system, extending the time an active agent is in touch with the dermis and boosting the penetration of active substances in a long-term, regulated way. These are its two major roles in cosmetic and cosmeceutical applications. Essential oils and active substances, including vitamins, antioxidants, and enzymes, can be found in cosmeceutical products made using chitosan (Abo Elsoud and el Kady 2019).

Chitosan and chitosan films can be utilized as sunscreens since their UV spectra show absorption below 400 nm. It has been observed that chitosan gel has an *in vitro* SPF of 0.89. When comparing two chitosan films, the shrimp chitosan film had a higher transmittance for UVA-UVB (300–250 nm) than the mushroom chitosan film did. This finding suggests that the UV resistance of mushroom chitosan extract was superior to shrimp chitosan film. The purpose of skin-conditioning products is to keep the skin in excellent condition. The only chitosan derivative recognized as a skin-cleansing agent is chitosan argininamide. Many chitosan compounds as well as certain chitin derivatives have been categorized as skin conditioning agents. Cosmetics known as humectants are used to enhance the water content of the skin's outer layers. Cationic humectants absorb to the skin's surface, changing it adversely. Chitosan and pyrrolidone carboxylic acid (PCA) were mixed to create a humectant that forms films (Morin-Crini et al. 2019a). To enhance the chitosan's humectant characteristics, acyl and alkylated chitosan derivatives were created. Using moisturizing lotions keeps the skin supple and smooth by increasing its water content. By oxidative degradation with H_2O_2 , many chitosan samples with varying molecular weight were created. The molecular weight and level of acetylation of the resultant chitosan affected their ability to absorb and retain moisture (Morin-Crini et al. 2019b).

A wide range of hair products, including shampoos, rinses, permanent wave agents, hair colorants, styling lotions, hair sprays, and hair tonics, include chitosan and its derivatives. In order to create clear, elastic films over the hair fibers, chitosan and its cationic derivatives interact with keratin. These films strengthen and soften hair while preventing breakage. Hyaluronic acid, collagen, and chitosan were combined, and the resulting films on hair were examined. The thickening of hair and improvement of its mechanical qualities as well as the hair's overall look and conditioning were caused by covering. In place of synthetic resins, alkyl-hydroxypropyl-substituted chitosan derivatives were added to hairsprays to prevent prolonged drying times, sticky hair, and helmet development (Aranaz et al. 2018). Chitosan's binding and film-forming abilities enable antioxidants to be administered to the skin gradually and steadily, improving the therapeutic efficacy of the resulting skincare formulation. As a result, chitosan serves as a dynamic agent for facilitating the delivery of medicinal chemicals and substances, such as nutrients, oxygen, antioxidants, and other substances, to the skin.

In Aquaculture

In recent years, the aquaculture sector has employed chitin and chitosan as feed supplements in the diets of fish and shellfish. Numerous studies have been done to determine how well chitin and chitosan work in the aquaculture industry. The scientific community is looking for safer and more environmentally friendly options to treat aquatic species due to the escalating limits on the use of antibiotics and other chemotherapeutics (Alishahi and Aider 2012). Utilizing immunostimulants to reduce aquatic creature illnesses is such a sustainable alternative strategy.

Chitin could be beneficial as a constituent of fish feed that has been specially prepared. There is proof that the gut bacterial population of fish and other aquatic animals differs noticeably from that found in the environment or in food. It has been discovered that adding chitin to feed for growing organisms enhances growth and feed conversion as well as stimulating the immune system by raising serum lysozyme levels, which also lowers production costs. However, significant fat accumulation in the liver, heart, and carcass occurs in some fish species due to chitin oversupplies (Ahmed et al. 2019). While chitosan, a deacetylated chitin derivative, was considered to be a more intriguing and effective bioactive polymer during the culture of particular farmed organisms, it was also examined as encapsulating bioactive chemicals. Vitamin C is better released while retaining its bioavailability when it is encapsulated in chitosan. Chitosan can encapsulate antigens for vaccinations and the virus that causes white spot syndrome, but how well they work depends on the infection. Additionally, chitosan has qualities that might help to enhance the quality of aquaculture effluent. The use of chitin and chitosan in aquaculture raises a number of unanswered concerns, including the stability of chitosan-encapsulated substances and the appropriate chitin content for each cultivated species, among others (Iber et al. 2021).

The research findings also suggest that the species being tested, the dose of chitin and chitosan, the time of administration, and the size of the chitin particle all have an impact on the immunostimulatory effects of these substances. Therefore, it is crucial to optimize the circumstances for the application of chitin and chitosan in order to both reap the most advantages and avoid any potential drawbacks, such as immune system suppression and growth retardation. The methods by which chitin and chitosan trigger immunological responses in fish and shellfish need to be better understood (Kamilya and Khan 2020).

In Fuel Cells

As a well-known source of clean next-generation energy, fuel cells draw interest. Solid fuel cells are one of them that are gaining popularity due to its mobility and stability. Therefore, it is necessary to discover novel fuel cell electrolytes that are inexpensive and have minimal environmental impact during the manufacturing process. Chitosan is an effective and ecologically friendly biopolymer that has recently undergone substantial research as a new material for use in fuel cells. Various fuel cells, including alkaline polymer electrolyte fuel cells, direct methanol fuel cells, and biofuel cells, can employ this biopolymer as both a membrane electrolyte and an electrode (Ma and Sahai 2013). Tissue-derived biomaterials

have recently attracted our attention as a fuel cell electrolyte due to their low cost and abundance in nature. Since chitosan's backbone contains free amine and hydroxyl functional groups, it can be chemically modified to suit a variety of needs. So, it can be used in fuel cell applications, pervaporation, ultrafiltration, electro-ultrafiltration, and polymer electrolyte membranes for the separation of metal ions, amino acids, and protein by adsorption.

Chitosan has a number of advantages for use as a polymer electrolyte membrane, including affordability and environmental friendliness, hydrophilicity, a useful quality for use in high temperature and low relative humidity environments, and low methanol permeability. Chitosan's main strength is that it has specific functional groups that enable chemical modification to tailor its properties (Shaari and Kamarudin 2015). Chitin functions as the fuel cell's electrolyte and transforms into a proton conductor. The quantity of water molecules grows along with the proton conductivity. In addition, the examination of proton conductivity in sheets and strongly oriented specimens revealed that chitin had anisotropic conductivity. It is discovered that the proton conductivity increases more along the chitin fiber direction than it does perpendicularly. The presence of proton conductivity in chitin is due to the proton transfer via the water network established between the chitin and water molecules, which is connected with the side chain of chitin along the direction of the chitin fiber (Muhmed et al. 2020). Numerous anionic and cationic chitosan-based membranes have undergone significant testing for use in fuel cells. In terms of proton conductivity, these chitosan-based membranes often do not provide appreciable benefits over conventional Nafion membranes, and as a result, the corresponding power density of associated fuel cells is likewise lower than that of Nafion-based ones. Therefore, for fuel cell applications, the inherent ionic conductivity of chitosan-based membranes needs to be further enhanced. Additionally, there is a need to improve the mechanical durability and shelf life of chitosan, which have not received much attention. Improvements to the chitosan membrane's qualities have been accomplished through chemical modification, the creation of chitosan blends, and composite materials. These techniques enhance some chitosan features while maintaining or enhancing others (Wan et al. 2006).

Other Applications

The excellent sorption capabilities of natural chitin and chitosan have been well documented in the literature. The creation of supports for chromatographic separation was a logical outcome of the sorption experiments. As chitin and chitosan are organic, renewing biopolymers with the additional advantages of being nontoxic, mechanically stable, and chemically inert, they have a great deal of promise for usage in industrial-scale separation processes. Due to the presence of a number of functional groups in the polymer chain unit, including an amine group at the C2 position, -CH₃ moieties in the acetamido group at the C2 position, and -OH groups at the C3 and C6 locations, they have been easily employed in liquid chromatography as a stationary phase. The primary method used in studies of chitin and chitosan was column chromatography, while studies of thin layer chromatography were rather few (only as chitosan-Fe for phenolic compound separation). A fascinating

alternative to column chromatography is membrane chromatography. This method is still in the early stages of research, but given how easily it can be scaled up for large-scale commercial separations with low pressure drop and high flow rates and simple membrane fabrication (Morin-Crini et al. 2019b).

The uses of chitosan in veterinary medicine, such as bone regeneration, analgesic and antibacterial properties, and wound healing, were previously discussed by different scientists. They explained well how chitosan may be used as a food additive and for the administration of drugs and vaccines to veterinary species. The delivery of chemotherapeutics like antibiotics, antiparasitic drugs, anesthetics, painkillers, and growth promoters to the mucosal epithelium for absorption for local or systemic activity, as well as the delivery of immunomodulatory agents to the mucosal associated lymphoid tissue for induction, are the veterinary drug delivery areas most likely to benefit from chitosan given the restrictions imposed by financial and animal restraint considerations (Gerdts et al. 2013).

Chitosan can be used in the wine-making process to clarify, deacidify, stabilize, and remove ochratoxin A, enzymes, and other unwanted compounds, such as metals and pesticides. Chitosan is also employed as a natural flocculant for the clarity of beer and an eco-friendly coagulant for the clarifying of passion fruit. Outlined recent chitosan-based matrices used for clarification, preservation, encapsulation, and active and intelligent packaging of various beverage types, including alcoholic, dairy-based, and nonalcoholic beverages, including fruit juices, nectars, concentrated fruit juices, tea, coffee, and tisanes (Galiano et al. 2018; Escudero et al. 2019). The most recent literature suggests that there is still a strong interest in using chitin-based materials, primarily chitosan, as a promising enzyme immobilization support for a variety of applications, ranging from the wine, sugar, and fish industries to sophisticated biosensors for both in situ measurements of environmental pollutants and metabolite control in artificial organs. These applications range from the removal of organic contaminants from wastewaters to the wine, sugar, and fish industries (Krajewska 2004).

In the papermaking sector, chitosan was first used in 1936, according to a report. The primary purpose was to increase the paper's moist strength. In order to build a cohesive, rupture-resistant film, chitosan functions as a substance that can interact with cellulose pulp to create paper. To help with compliance with environmental requirements, this biopolymer is also nontoxic, biodegradable, and environmentally friendly. In order to remove lignin, color, and unwanted pollutants from pulp and paper wastewaters as well as reduce total organic carbon and chemical oxygen demand, chitosan is also utilized in this process. The use of nanoparticles and nanostructured materials in papermaking has recently been studied. The chitosan market is anticipated to expand quickly as a result of rising usage in a variety of industries, including water treatment, the beverage sector, nutrition, and cosmetics. Indeed, the markets for nutraceuticals and cosmeceuticals are expanding (Gassara et al. 2015). Chitosan is also in higher demand in developing nations. Products that are therapeutic and biomedical are also anticipated to positively affect the industry. It is significant to note that chitosan applications in the biomedical field are still limited, despite the fact that numerous papers and patents have been reported over

the past 20 years. This is primarily because it is extremely challenging to access the biopolymer with sufficient purity and dependability at its source.

Conclusions

Chitin and chitosan are miraculous natural biopolymers found in the cell walls of fungus, the exoskeletons of insects and mollusks, and the shells of crustaceans. They are manufactured commercially from crab shells and have several uses in agriculture, medicine, and other fields.

It is a strong chemical of interest, because it is a naturally biodegradable, environmentally friendly, biocompatible, and nontoxic substance. To various degrees, chitin is deacetylated to create chitosan. Chitin can be produced via chemical or biological processes. Chitosan, on the other hand, is a substance with several potential uses. Almost all biological disciplines can benefit from the use of chitosan. Since chitosan has strong antibacterial qualities, it may be used to make antimicrobial compounds for use in food, farming, and medical procedures. Chitosan has a number of incredibly intriguing qualities, such as biocompatibility and antibacterial activity, but its use in the biomedical field is still rather restricted. This is mostly because it is exceedingly challenging to get enough purity and source reliability for the biopolymer.

Cross-References

- ▶ [Biopolymer-Based Composites](#)
- ▶ [Biopolymer Films and Composite Coatings](#)
- ▶ [Nanofibers and Nanomembranes of Biopolymers](#)

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Sustainable Biodegradation and Extraction of Keratin with Its Applications 26

Shinar Athwal, Swati Sharma, Shreya Gupta, Ashok Kumar Nadda, Arun Gupta, and Mohamed Saad Bala Husain

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S. Athwal · S. Sharma (✉) · S. Gupta
University Institute of Biotechnology, Chandigarh University, Mohali, Punjab, India
e-mail: swati.e8750@cumail.in

A. K. Nadda
Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat, Solan, Himachal Pradesh, India

A. Gupta · M. S. B. Husain
Faculty of Chemical and Process Engineering Technology, Universiti Malaysia Pahang, Gambang, Pahang, Malaysia

Abstract

Keratin has been gaining wider acceptance as an efficient biomaterial for application in various sectors. The extraction and degradation process of keratin is the primary and significant aspect of keratin utilization as a biomaterial. This review comprises sustainable degradation of keratin via microbes, fungi, and green solvents, followed by sustainable extraction of keratin, and lastly, the applications of keratin in several areas have been discussed. RCS6 a yeast strain, *Fusarium* species 1A, *Bacillus* species 8A6, *B. subtilis* 8 are the microbes, while NMMO a sustainable solvent are discussed for degradation of keratin. Further, Green phenomenon to extract keratin proteins are with liquid deep-eutectic solvent (urea-sodium acetate), keratin regeneration via sustainable solvent composed of L-cysteine and lactic acid, sustainable medium of the hydrated ionic solution to extract keratin, efficient extraction of keratin via choline chloride and oxalic acid, and keratin extraction via novel ultrafiltration process is mentioned as sustainable keratin extraction processes. Lastly, the possible and potential applications of keratin in areas of bioplastics, cosmetics, tissue engineering, drug release, wound healing, and other industries have been discussed.

Keywords

Keratin · Keratinase · Extraction · Applications · Bioplastics · Biomedical

Introduction

Keratins are abundantly available and insoluble structural proteins exhibiting high levels of cross-linking, which are the major constituents of strong tissues, like a feather, wool, horn, nail, hair, etc. (Bhange et al. 2016). Since keratin is a naturally derived polymer and possesses physiological characteristics like biocompatibility, toughness, biodegradability, cell proliferation, and attachment thereby suggesting keratin as a potential biomaterial for several applications in tissue engineering, bio-packaging, cosmetics, and industries, etc. (Placone et al. 2017; Aggarwal et al. 2020; Singh et al. 2022; Kakkalameli et al. 2022). The keratin waste is a prominent resource for renewable raw substances, as discarded organic waste material derived from poultry feathers, hair, horns, discarded wool, and keratin waste from leather/textile industries accounts for five million tons of waste at annual basis which can be considerably utilized to develop environment-friendly biomaterial with preferable applications in various fields (Wang et al. 2016a; Sharma et al. 2017; Sharma and Gupta 2016) (Fig. 1). The extraction of keratin is the primary and essential step for the employment of keratin as a biomaterial (Kamarudin et al. 2017; Kumawat et al. 2022). Alkaline extraction, ionic liquids, oxidation, reduction, and DES are some of the prominently used keratin extraction techniques. The keratin extraction via alkaline method requires more quantity of alkaline reagent to neutralize acid thereby influencing the content of amino acids leading to decreased keratin yield. The

Fig. 1 Sources of keratin

reduction method on the other hand preserves the keratin chain framework but exhibits noxious and harmful effects. The oxidation process is convenient to conduct but partial oxidation of cystine to cysteic acid occurs by performin or peracetic acid. Ionic acids and DESs are sustainable solvents demonstrating eco-friendly properties with the relevance of a good yield of keratin (Zhang et al. 2021). DESs have to gain more popularity in comparison to ionic liquids as the ILs have few drawbacks like longer dissolution time, low solubility, high price, etc. (Zhang et al. 2021). Various DESs utilized for keratin extraction are, viz., urea-sodium acetate (90% wt.) in 2:1 molar ratio (Nuutinen et al. 2019), lactic acid, L-cysteine (Shavandi et al. 2021), hydrated ionic solution (Singh and Prasad 2019), and choline chloride and oxalic acid. Other sustainable approaches for keratin extraction were novel ultrafiltration phenomenon (Cassoni et al. 2018). Moreover, keratinolytic microbes, as well as sustainable solvents (NMMO) (Ma et al. 2017), have been reported for the biodegradation phenomenon of the keratin protein so as to reduce the keratin waste and also to remarkably degrade keratin at a large-scale (Gupta et al. 2022a). The extracted and degraded keratin can be possibly employed for applications in the fields of bio-packaging, tissue engineering, agriculture, cosmetics.

Structure, Classification and Properties of Keratin

Keratin is grouped into two categories, viz., β -keratin mostly present in feathers and epidermis and α -keratin mostly present in hair (Deb-Choudhury et al. 2016). The keratin is composed of long amino acid polymerized chains. The β - sheets and

α -helix structures occur because of continuous twisting of polypeptide chain backbone owing to hydrogen bonds between amino groups and carboxyl groups within the peptide chain (Shah et al. 2019). The polypeptide chains in α -helix forms hydrogen bonding with maximum possible interactions between amino groups of one amino-acid and the carbonyl group of another amino-acid. The α -helix structure exhibited a molecular weight of 40 kDa–68 kDa and a diameter of 7 nm–10 nm (Wang et al. 2016b). While β -sheet structure exhibits inter-molecular hydrogen bonds between the amino and carbonyl group and has a molecular mass around 10 kDa–22 kDa with a diameter of 3 nm–4 nm (Chen et al. 2012). Keratin protein is a polypeptide molecule composed of several amino acids having inter-molecular bonding of disulfide-cysteine amino acids along with intra-/inter- molecular bonding of polar and non-polar acids. Keratin are water in-soluble as well as in-soluble in various weak alkaline, acids solutions and organic solvents with resistance to protein-digesting enzymes (trypsin or pepsin). Keratin has low contents of methionine and histidine while have high contents of proline, serine, cystine, and glycine with no or few traces of tryptophan. The cystine significantly specifies the physiochemical properties exhibited by the protein. In comparison to other proteins, keratin have lower solubility and higher stability owing to intra- and inter-chain cysteine disulfide cross-links. Also the other bonds like, ionic, hydrophobic, and hydrogen bonds play a vital part in the properties and stability of keratin. The availability of ionic bonds is dependent on the pH and is significantly high at isoelectric-point ($\text{pH} = 4.9$) which is the zwitterion form of protein ($+\text{H}_3\text{N}-\text{CHR}-\text{COO}-$), whereas under extreme basic and acidic conditions, lowest levels of ionic bonds are present. As a result ionic bonds get reduced via carboxylic groups protonation at lower pH value as well as amine group deprotonation at higher pH value. On the other hand, disulfide bond gives keratin higher resistance toward enzymatic and chemical reactions and provides compact 3-dimensional composite. Based on sulfur content keratin can be soft (50–75% moisture and 2% sulfur) or hard (4–8% sulfur) (Shavandi et al. 2017). As a result, the keratin as a component offers remarkable parameters to be employed as an efficient biomaterial.

Biodegradation of Keratin via Sustainable Approach

The keratinases are peptidases that exhibit the property of keratin degradation and have been prominently described for various bacterial species (Daroit and Brandelli 2014) as well as for dermatophytic fungal species (de Medeiros et al. 2016). One such example of fungal keratin degrading species is anamorphic basidiomycetous yeasts of the *Trichosporon* genus. The availability of keratinolytic species in the tannery waste exhibits significant potential for the bioremediation of contaminated water and soil as well as acts as a resource for efficient biodegradable enzymes to lessen and dehair the effect of the tannery phenomenon (de Medeiros et al. 2016). de Medeiros et al. conducted research to extract and examine keratinolytic yeasts that generate enzymes that can possibly replace a few chemicals reagents utilized for the tanning process during leather production. RC-S6 a yeast strain was putatively

recognized as a strain of *Trichosporon* Loubier on the basis of ribosomal and biochemical DNA analyses (de Medeiros et al. 2016). The strain generated sulfide and rapidly degraded nail keratin at a rate of 193.3 U ml^{-1} . Moreover, gelatinase and keratinase examination demonstrated that the enzymes released via RC-S6 preferably degraded keratin and sulfitolysis is an essential phenomenon for the better action of keratinases/peptidases over keratin filament (Cedrola et al. 2012). Thus, the keratinases from RC-S6 strain have potential abilities for keratin degradation. The keratin waste hydrolysis via microbes is regarded as a good biotechnological alternative to valorization and recycling (Lange et al. 2016). In spite of the resistant structure of keratin wastes, it can be efficaciously degraded with various microbes via keratinase secretion, which is a promising enzyme for different applications and operations such as fertilizers, detergents, and textile and leather industries (Brandelli 2008). With the approach to separate keratinolytic microbes which can possibly reach the commercial application as keratinase producers, Calin et al. assessed the dynamics and phenomenon of the keratin biodegradation with various strains of keratinolytic fungus separated from the soil sample (Călin et al. 2017). The capability of the fungal species to bring about keratin degradation was estimated by Fourier Transform infrared spectroscopy, i.e., FTIR, Scanning Electron Microscope, i.e., SEM, and the thermogravimetric analysis, i.e., TGA. The observations via SEM revealed information about the interactions between structural composites of hair filaments and microbes. On the other hand, FTIR bands spectra at $1035\text{--}1075 \text{ cm}^{-1}$ brought about the appearance of sulfoxide bonds owing to the breaking of S-S bonds, which showed the initiation process of keratin protein biodegradation. As per the TGA analysis, in the zone second of the thermal denaturation, i.e., during keratin degradation, the specimen incubated in *Fusarium* species 1A exhibited the most loss of weight of 71.10%. Therefore, among the strains examined, *Fusarium* species 1A was significantly the most powerful organism during the degradation phenomenon with the active denaturation process of the polypeptide chains. Thereby, *Fusarium* species 1A was chosen as the efficient keratinolytic microorganisms with economic and scientific capabilities (Călin et al. 2017).

The first *Bacillus* sp. with the capability of degrading keratin was isolated by Molyneux (Molyneux 1959). The *Bacillus* species like *B. cereus*, *B. licheniformis*, and *B. subtilis* are commonly known organisms for their keratinolytic abilities (Huang et al. 2020). Huang et al. studied the efficiency of *Bacillus* species 8A6 as a keratin degrader and belonged to the *Bacillus pumilus* species (Huang et al. 2020). On the basis of the comparison between the whole genome, *B. 8A6* was significantly related to the *Bacillus safensis* which also has been reported to exhibit keratin degradation properties. The *B. 8A6* exhibits the parameter of degrading keratin present in feathers after the growth period of 24 h. Moreover, *Bacillus 8A6* culture broth's proteomic examination demonstrated more availability of proteins exhibiting peptidase activity functions. *Bacillus 8A6* showed the expression of a dipeptide, five proteases, and four oligopeptide binding proteins. Furthermore, the keratin of hooves, bristles, and feathers is rich in cysteine-related re-calcitrant protein system (Huang et al. 2020). After the degradation process of hooves, bristles, and feathers via keratinolytic proteases, the resultant oligopeptide which is cysteine-rich can be

preferably used for the generation of glutathione and the glutathione can be hydrolyzed into cysteine-glycine and glutamate which can be directly used by the cells of microbes (Huang et al. 2020; Strasser et al. 2015). Thereby it was reported that along with the T3, M12, and S01A families of bacterial proteases, the S08 family is also involved in the keratin degradation process (Huang et al. 2020). Likewise, He et al. reported the process of keratin degradation via *B. subtilis* 8 (He et al. 2018). The microbe degraded the keratin after a time duration of 48 h. The cystathionine gamma-synthase produced by *B. subtilis* 8 degraded the keratin present in the feather. The percentage degradation of the feather was estimated through weightlessness. The feathers residues in the media following the fermentation process were filtered via speed-filter paper (He et al. 2018). The Keratinase (Gradišar et al. 2005), i.e., KT, disulfide bond-reducing (Prakash et al. 2010) (DRT), and feather weight-loss, i.e., FWS enzyme activities were estimated after fermentation process for 12 h, 24 h, 36 h, 48 h, 60 h, and 72 h. All the three parameters progressively enhanced after 36 h of the fermentation process and couldn't be spotted by naked eyes. FWS attained the maximum value of 83.7% after 60 h of fermentation, thereby indicating complete degradation of feather via *B. subtilis* 8 while the KT attained maximum value at 48 h of 21.6 U/ml. The DRT values attained maximum value at 24 h of 17.4 U/ml. The barbules were degraded completely, leaving behind the feather shaft and residual pinnule after 24 h of fermentation. Moreover, significant feather shaft degradation was observed after 32 h of incubation. After a duration of 48 h, the structure of the feather shaft initiated to collapse with proper and complete degradation at 48 h. During the process of fermentation, 17 amino acids, involving 5 essential amino acids, were generated in the suspension. The concentration estimated for all-total amino acids was around 34.189 nmol/ml (He et al. 2018). Thus, *B. subtilis* 8 can be significantly employed for keratin degradation even at a large scale. An environment-friendly mode of keratin extraction was offered by Ma et al. through the utilization of N-methyl-morpholine N-oxide, i.e., NMMO, a sustainable solvent (Ma et al. 2017). The authors added and dissolved feathers into 50% of NMMO at 110 °C under the influence of mechanical agitation provided with decreased distillation pressure. The feather progressively dissolved after the removal of 100 g water, which signified that the feather could be perfectly dissolved into 75% NMMO suspension. The dissolution phenomenon is eco-friendly and efficacious in comparison to oxidation and reduction processes which involve more use of chemical reagents. The addition of extra water increased the dissolution of keratin as water acts as an anti-solvent for keratin. Further, precipitation of keratin was brought about by the addition of HCl after the centrifugation process. The cross-linkages along with disulfide bonds were ripped during keratin degradation at 100 °C which possibly degraded in to water-soluble polypeptide which was supported by the analyzed outcomes of size-exclusion HPLC for filtrate and amino acid examination where the estimated amount of polypeptide and cystine infiltrate was around 63.7% and 0.34%, respectively (Ma et al. 2017). Thus, NMMO suspension can be considerably utilized as a green solvent to degrade as well as extract keratin proteins from feathers (Fig. 2).

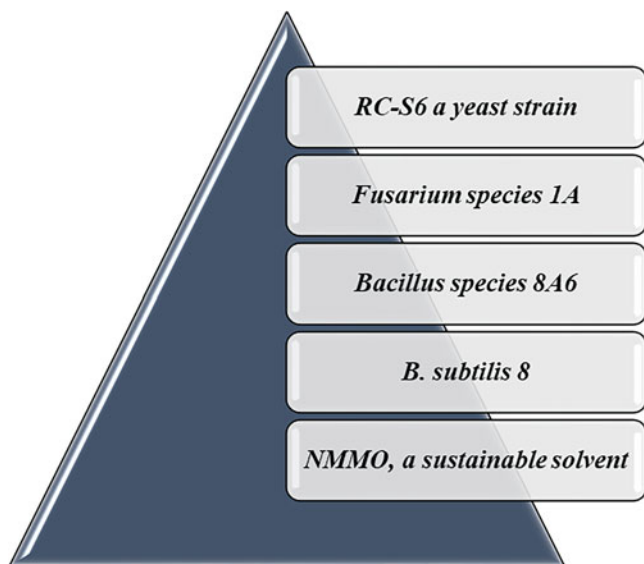


Fig. 2 Discussed biodegradation approach for keratin protein

Extraction of Keratin

Green Phenomenon to Extract Keratin Proteins with Liquid Deep-Eutectic Solvent (Urea-Sodium Acetate)

A huge amount of feathers in form of waste are generated in the poultry industries which are re-cycled, incinerated, or dumped in landfills (Nuutinen et al. 2019). However, the green extraction of protein, i.e., keratin from the feathers provides a more convenient and sustainable approach toward the handling of the disposed feathers. Deep-eutectic solvents or DESs provide a green platform to extract keratin protein from the feathers. Abbott et al. primarily presented DESs as a cost-effective and convenient alternative to ionic liquids (Abbott et al. 2004). DESs possess the properties such as biocompatibility, biodegradability, and non-toxicity which enhances its use as a green solvent to extract keratin. The DESs are acquired when the constituents are mixed properly in the appropriate molar ratio via which the system attains its least melting point (M.P.). The M.P. is decreased owing to the interactions between constituents via hydrogen bonds which reduces the system's lattice energy (Dai et al. 2013). In general, DESs are made of solid constituents which altogether developed a mixture having lower M.P. in comparison to individual constituents (Smith et al. 2014).

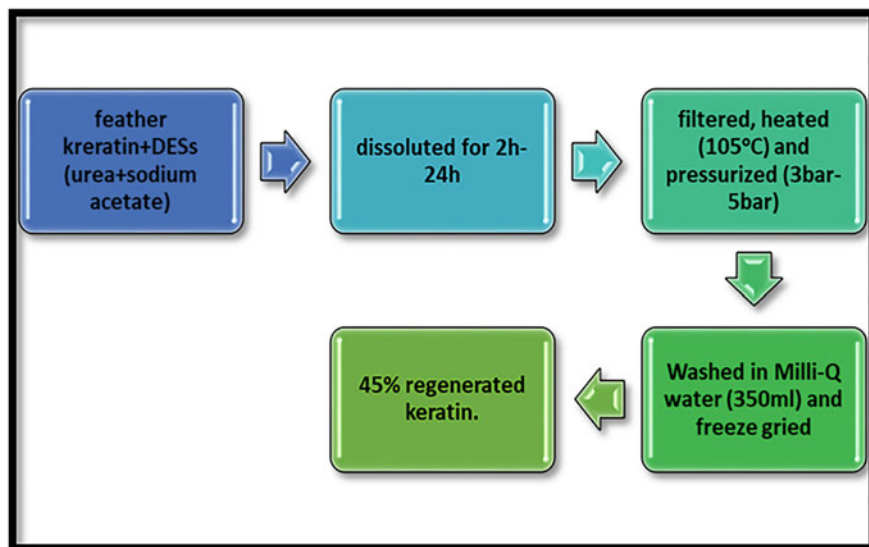


Fig. 3 Green phenomenon to extract keratin proteins with liquid deep-eutectic solvent (urea-sodium acetate)

Nuutinen et al. proposed the use of DESs to process the feathers with a liquid DES solvent to generate uniform feedstock of keratin (Fig. 3) (Nuutinen et al. 2019). The utilized DESs consisted of urea-sodium acetate (90% wt.) in a 2:1 molar ratio. Following the treatment with DESs, water was utilized to dilute constituents of DES and thereby revive a dissolved form of keratin. The optimum conditions for processing were based on the yield of keratin and properties with varied timings of dissolution varying between 2 h and 24 h with temperatures between 80 °C and 100 °C. Moreover, thermal specifications of the utilized DES were obtained by using thermogravimetric analysis and differential-scanning-calorimetry. The 6-h dissolution time led to the 45% regeneration of the yielded keratin and caused 86% of feathers to be dissolved. The regenerated keratin via the experiment predicted that a higher dissolution temperature as well as longer dissolution time, decreased the total content of sulfur and disulfide with increased ranges for molecular weights while the crystallinity and few ordered secondary systems were lost. The structural variation of the re-generated keratin and dissolution of the keratin occurred owing to the capability of liquid DES to alter the interactions in the keratin of feather causing the cleavage of disulfide bonds as well as partially breaking keratin's polypeptide backbone. Thus, the characteristics and yield of keratin can be preferably optimized by tuning the molar ratio and dissolution parameters of the DES (Nuutinen et al. 2019).

Keratin Regeneration via Sustainable Solvent Composed of L-Cysteine and Lactic Acid

Production of waste wool accounts for approximately >10,000 tons annually (Shavandi et al. 2021). The waste wool is composed of 95% keratin by weight (Su et al. 2020) which can be regenerated conveniently by using sustainable solvents like L-cysteine and lactic acid. L-cysteine was used as a sustainable reducing agent by Wang et al. for dissolution of wool in which L-cysteine was combined with concentrated urea solution of 8 M, with pH value >10 by utilizing NaOH, resulting in 72% dissolution after heating for 5 h at 75 °C (Wang et al. 2016a). Lactic acid, on the other hand, makes the stratum corneum soft in order to absorb polar groups exhibited by chains of keratin and further decrease interactions between the chains without enhancing the content of water within the stratum corneum (Shavandi et al. 2021).

Shavandi et al. employed a novel sustainable solvent composed of lactic acid and L-cysteine to regenerate keratin from wool (Fig. 4) (Shavandi et al. 2021). Primarily the authors defatted wool via Soxhlet extraction by using hexane/dichloromethane v/v in a 1:1 ratio for a time period of 16 h. Then 0.4 g of defatted and dried wool was combined and treated with 2 g of L-cysteine and 20 mL of lactic acid at 95 °C for a time period of 3.5 h. The sustainable solvent destabilized the macromolecular systems by losing intra and inter molecular forces and breaking of disulfide bonds enhances the dissolution and extraction of keratin from the wool without any damage to the peptide bonding (He et al. 2020). Moreover, the strength and stiffness of wool are because of various inter- and intramolecular bonds of disulfide owing to more

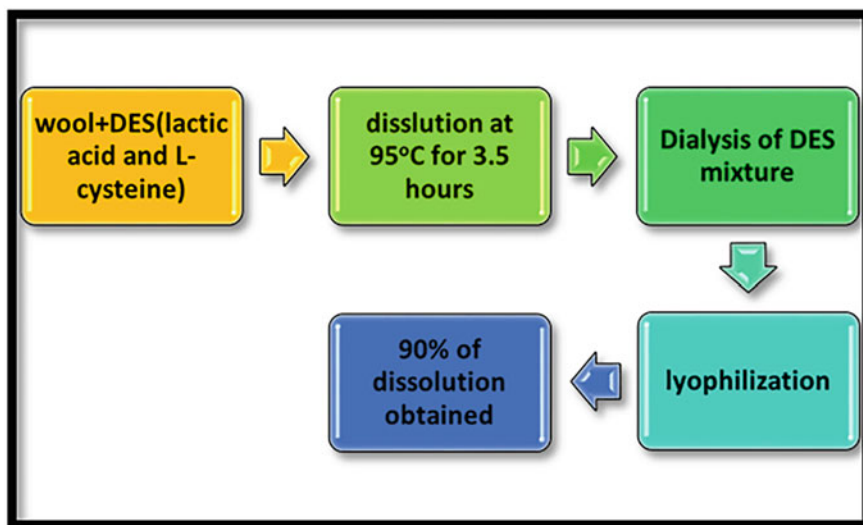


Fig. 4 Keratin regeneration via sustainable solvent composed of L-cysteine and lactic acid

amount of cysteine residues within the polypeptide backbone. Heating of wool in the availability of lactic acid causes the crystalline compact system of keratin to denature thereby leading to the formation of an amorphous structure with softened and swollen wool which enhances the penetration power of L-cysteine as well as leads to the exposure of disulfide bonding toward L-cysteine which subsequently decreases disulfide bonds and increases wool dissolution. The regenerated keratin exhibited β -sheets while the α -helix disappeared. Furthermore, keratin was extracted after the dialysis process of the sustainable solvent followed with lyophilization. Also, by employing the combined mixture of L-cysteine and lactic acid didn't require regularly used chemicals like sodium hydroxide, urea, and sodium sulfite. However, the solubilization phenomenon of keratin still requires optimization for processing parameters like ratio of liquid to solid, time and temperature for processing. Thus, lactic acid and L-cysteine mixture can be preferably utilized as sustainable solvent for the regeneration of keratin protein without the loss of peptide chains (Shavandi et al. 2021).

Employing Sustainable Medium of the Hydrated Ionic Solution to Extract Keratin

The ionic liquids or ILs are one of the convenient and suitable solvents for processing different biopolymers such as proteins like keratin (Schröder 2017). ILs are standardly utilized to produce various chemicals and to treat lignocellulose biomass. The treatment of lignocellulose biomass led to the high production of glucose (Xu et al. 2016). The imidazolium composed ILs have been reported to be used for keratin dissolution at a high temperature of 130 °C and longer time duration. On the same line, 1-butyl-3-methyl-imidazolium chloride composed ILs was employed for the dissolution of keratin present in human hair. Likewise, dimethyl phosphate composed ILs efficiently extracted keratin from wool at a reduced dissolution time duration (Singh and Prasad 2019).

Singh et al. compared the activity of dissolution among three different aqueous ILs: tetrabutyl ammonium bromide, i.e., TBAB; tetrabutyl ammonium acetate, i.e., TBAA, choline hydroxide; and tetrabutyl ammonium hydroxide, i.e., TBAH, all in the same proportions in water (Fig. 5) (Singh and Prasad 2019). The authors observed that TBAH solubilized 25% (w/w) of the human hair, choline hydroxide solubilized 10% (w/w) of human hair, while the other ILs didn't solubilize the human hair at room temperature. Moreover, the dissolution of human hair with TBAH for 9 h caused the complete dissolution of human hair and didn't show the availability of insoluble fibers of human hair when the aliquots were observed under optical light microscopy. Further, the water was evaporated from the solution containing keratin to get a viscous suspension, and acetone addition to the suspension caused the precipitation of opaque or white-colored crude keratin. The extracted keratin was separated via centrifugation process and then washed with the acetone solution and finally placed in a desiccator under decreased pressure conditions in order to procure dry keratin powder. The IL which was left behind following the

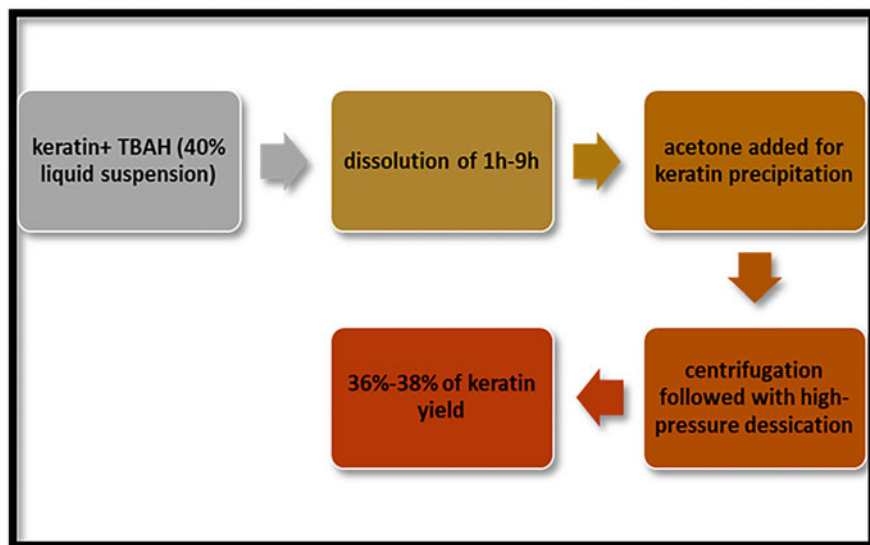


Fig. 5 Employing sustainable medium of the hydrated ionic solution to extract keratin

separation of protein demonstrated a good amount of nitrogen which could be utilized to fortify an aqueous seaweed plant bioreactor that exhibits low nitrogen concentrations. Furthermore, The, α - helix structure and β -sheet structure of keratin protein of human hair were not altered following the regeneration of keratin thereby indicating stability exhibited by the keratin structures even after dissolution within aqueous ILs. The value of E-factor (Paquin et al. 2015) calculated for the keratin regeneration via ILs was 4.25×10^{-2} , which specified that an almost negligible amount of waste was formed during the extraction phenomenon thereby demonstrating the regeneration process via ILs to be sustainable and green in nature (Singh and Prasad 2019).

Efficient Extraction of Keratin via Choline Chloride and Oxalic Acid

The combination of choline chloride, i.e., ChCl, and oxalic acid, i.e., OA, forms an efficient DES system. The DES is particularly a solvent platform prepared through the complexation phenomenon of the hydrogen bond donor and the hydrogen bond acceptors (Abbott et al. 2003). The development process of a DES is important and it is crucial to develop a significant dissolution platform as per the objective and purpose of the required action. When various types of hydrogen donors are attached with the hydrogen acceptors, the efficiency of dissolution exhibited by the DES has also changed accordingly (Zhang et al. 2021). For instance, Wang et al. employed urea, citric acid, oxalic acid, and hexadiol as hydrogen donors and ChCl as a hydrogen recipient to examine the dissolution for the DES developed for the hair

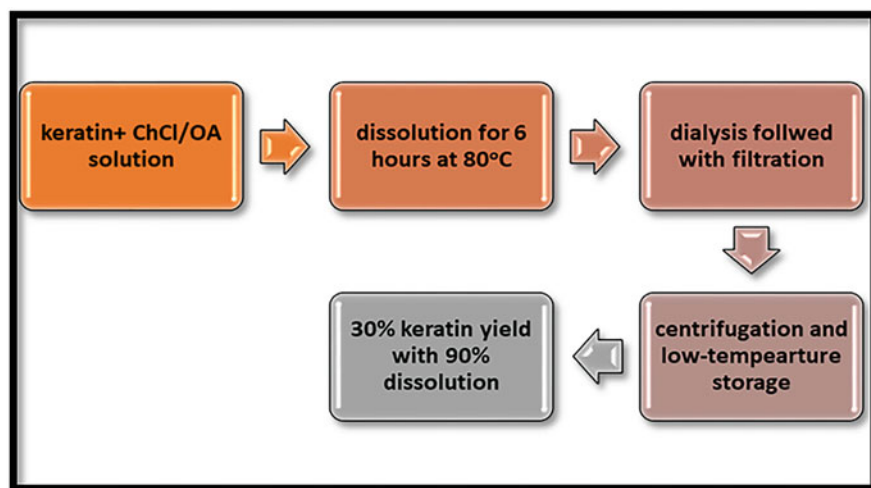


Fig. 6 Efficient extraction of keratin via choline chloride and oxalic acid

of rabbit and concluded that ChCl and OA exhibit the best dissolution effect (Wang et al. 2018).

On the same line, Zhang et al. employed various molar ratios of ChCl and OA to regenerate keratin from feathers (Fig. 6) (Zhang et al. 2021). The authors combined ChCl with OA with a specific molar ratio along with an appropriate proportion of water to develop a dissolution platform. The feathers were completely dissolved into the dissolution platform to procure the extracted keratin, which exhibited water-insoluble and soluble fractions which were segmented via filtration process. The feathers were placed into various dissolution platforms for 4 h at 90 °C to examine the effect of the different compositions of the developed dissolution platforms over the dissolution of feathers. The ChCl/OA molar ratio greatly influenced the dissolution efficacy of the feathers. As, when the ChCl/OA molar ratio altered from 3:1 molar ratio to 1:3 molar ratio, the suspension color intensified progressively from amber color to dark brown color, which can be owing to the liberation of melanin protein between the cellular bodies of feathers cortex at the time of dissolution. Moreover, the rate of dissolution enhanced gradually, which was because of the enhanced concentration of OA within the dissolution platform as well as the increased concentrations of free available hydrogen protons within the platform (Zhang et al. 2021). The free available hydrogen protons could develop strong bonds with amino and imino groups in the keratin, thereby increasing the possibility for large concentrations of OA to be significantly attracted toward protein and consequently deconstructing the feather by breaking the bonds of hydrogen between and within the composites of keratin (Bai et al. 2017). Further, the increased proportions of OA slowed down the enhancement rate of dissolution. The estimated rate of dissolution for ChCl/

OA (1:3) and ChCl/OA (1:2) was 98.75% and 97.89% respectively, with no considerable difference. While, the increased content of OA in DES, increased the acidity of the dissolution platform gradually, which intensified the degradation process of keratin resulting in keratin with less molecular weight. Thus, ChCl/OA with the molar ratio of 1:2 is a more convenient process condition (Zhang et al. 2021).

Keratin Extraction via Novel Ultrafiltration Process

A new environment-friendly process for keratin extraction from hair was developed by Cassoni et al. (Fig. 7) (Cassoni et al. 2018). The authors employed commercially available detergent related to the class of degreasers, capable for efficacious and rapid dissolution of hair, and subsequently followed by a standard filtration process to eliminate residues present from the trimmings of skin with lard and fat (Cassoni et al. 2018). The obtained solution was then processed via ultrafiltration process to attain a suspension with increased purity and protein content. The digestion and cleaning steps at optimized conditions of temperature and time (6 h) with the standard Mistolin solvent resulted in a suspension with nil hair fibers thereby depicting complete dissolution. Mistolin is a biodegradable solvent reagent with biodegradability >90% and is prominently utilized by food-related industries. The resultant final product is turned into brown liquid by the Mistolin with 12.5 pH owing to NaOH; however, products comprising of NaOH do not bear adverse impact over the environment (USEPA 1992). Moreover, the subsequent filtration process removed solid residues and fat present within the digested suspension. Small residues were present in the brown-colored filtered suspension which was eliminated later via centrifugation or microfiltration process

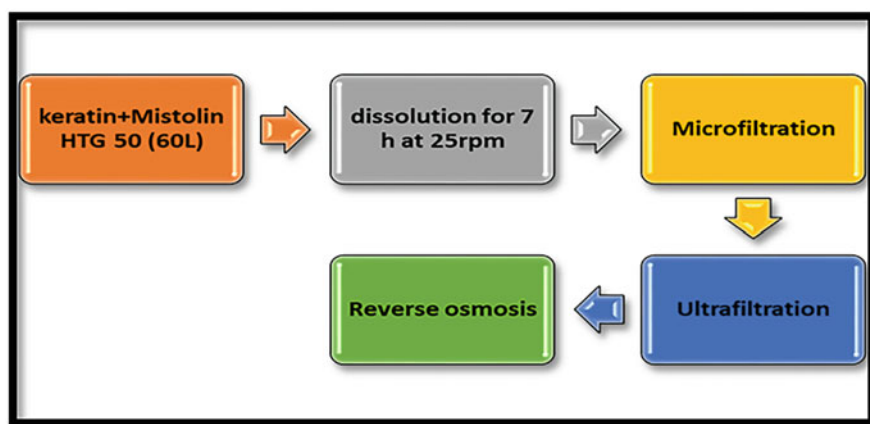


Fig. 7 Keratin extraction via novel ultrafiltration process

while the suspension obtained after the precipitation process was green colored with small residues. Further, the ultrafiltration process executed at the laboratory level gave a satisfactory yield of protein. In addition, executing ultrafiltration at the pilot-scale level produced a decreased protein yield owing to protein loss during the microfiltration method. The utilization of organic membrane can enhance the extraction process of keratin at pilot scale and could possibly be purer in form. The utilization of ultrafiltration method, is an economical membrane separation platform employed to sustain macromolecules, which have more-throughput of product, convenient to level up for industrial generation (Baldasso et al. 2011; Saxena et al. 2009). The membranes of ultrafiltration are reusable thereby decreasing the production cost of keratin. Furthermore, ultrafiltration decreases the time for execution and makes the extraction process convenient as the process can extract keratin within 9 h of duration. Also, solvent recovery is possible as well as significant optimization for the cleaning phenomenon of the solvent could be prepared to eliminate dust particles as a result of the digestion process. Therefore, the standard protocol followed for keratin extraction by novel ultrafiltration method was the digestion of hair (pig) by Mistolin followed by a filtration process, continued with centrifugation at laboratory level and microfiltration at pilot scale level, subsequent treatment under ultrafiltration process with reverse osmosis being optional at pilot scale and ultimately procuring keratin suspension (Cassoni et al. 2018).

Comparison Among the Discussed Extraction Methods

The first method, i.e., Green phenomenon to extract keratin proteins with liquid deep-eutectic solvent (urea-sodium acetate) exhibited 45% of keratin yield with 86% of keratin dissolution after 2–4 h of dissolution process. The next method, keratin regeneration via sustainable solvent composed of L-cysteine and lactic acid demonstrated 90% of keratin dissolution after 3.5 h (a time period less than that of first method) at 95 °C. The third method, employing sustainable medium of the hydrated ionic solution to extract keratin, revealed 36%–38% of keratin yield after 1–9 h of dissolution process but unlike DESs ionic solutions are toxic, expensive, and difficult to prepare. The fourth method, efficient extraction of keratin via choline chloride and oxalic acid depicted 30% keratin yield with 90% dissolution after 6 h of dissolution process at 80 °C. The last method, Keratin extraction via novel ultrafiltration process suggested 50% keratin yield after 7 h of dissolution at 25 rpm. Of all the discussed methods, second process didn't require extra solvents and had less dissolution time for keratin extraction. In case of large-scale extraction process, the ultrafiltration method is the most suitable one with highest keratin yield of 50%. For small-scale keratin extraction L-cysteine and lactic acid can be used owing to less dissolution time and cost-effectiveness (Fig. 8).

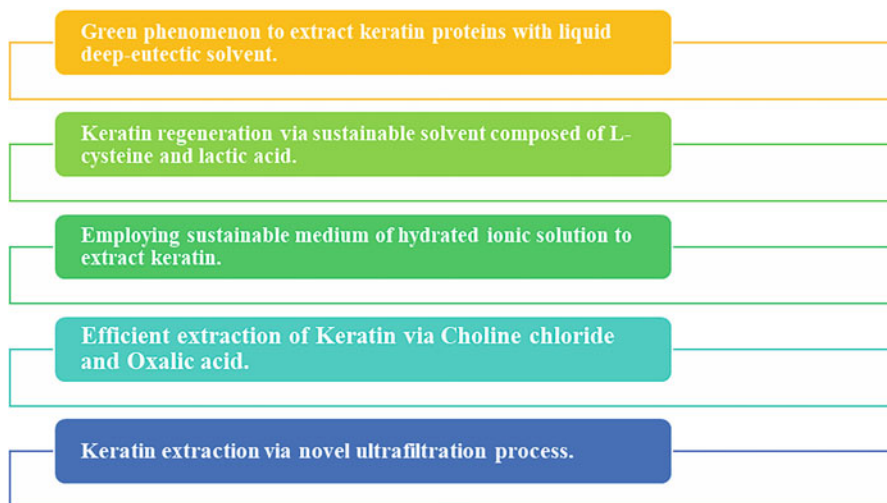


Fig. 8 Discussed sustainable extraction approach for keratin protein

Applications of Keratin

Keratin and Bioplastics

The bioplastics are the perfect alternative to normal plastic composites, demonstrating eco-friendly characteristics. Keratin-based bioplastics are one of the efficient construction materials for the development of bioplastics as employing keratin can lead to decreased keratin waste in the environment and subsequent generation of bioplastics with environment-friendly parameters (Alashwal et al. 2020; Sharma et al. 2018; Gupta et al. 2022b). Various studies have been conducted for developing keratin-based bioplastics. For instance, Dou et al. extracted hydrolyzed keratin feather from chicken feathers by utilizing the economical process of acid precipitation and alkaline extraction equipped with sodium sulfide and urea treatment with the aim of developing and characterizing environment-friendly films dependent over hydrolyzed keratin feathers with different glycerol concentrations via the thermoplastic method (Dou et al. 2016). Glycerol with lower molecular mass and convenient diffusion properties with the polar molecules of protein acts as an efficacious plasticizer for developing protein-dependent materials. The thermal examination revealed that high pressure and high temperature enhanced the relative compatibility between molecules of hydrolyzed feather keratin and glycerol and also that the water addition during the hot-pressing phenomenon of films is a necessary factor. Moreover, the glass-transition temperature of hydrolyzed keratin feather and glycerol mixture reduced as the concentration of glycerol increased which could be because of the development of hydrogen bonds between keratin composites and glycerol, thereby losing internal interactions within the protein matrix. Moreover, the glycerol

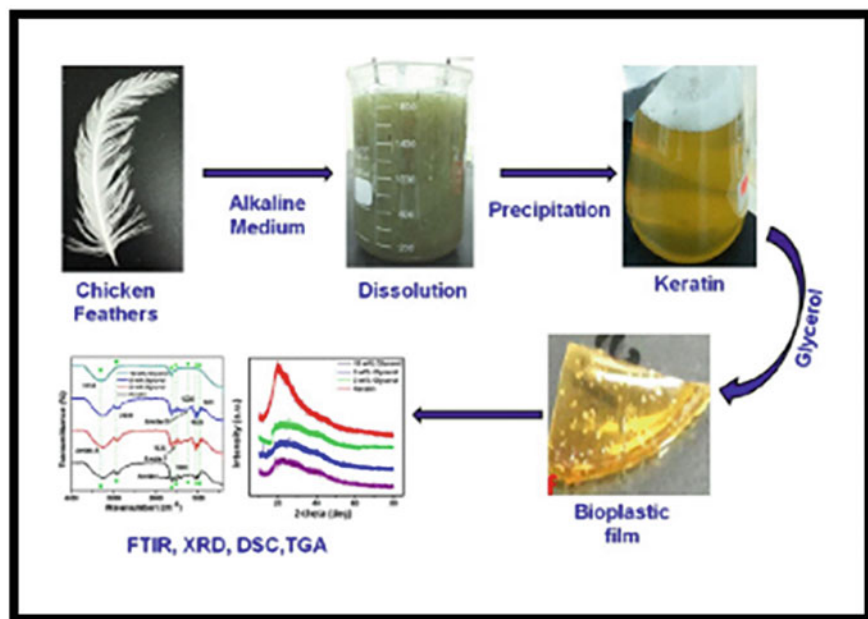


Fig. 9 Graphical representation for the development of keratin based bioplastics (Ramakrishnan et al. 2018)

concentration considerably impacted the water resistance, tensile properties, water barrier factors, and microstructure of the hydrolyzed keratin feather, hence enhancing the commercial applications of the developed bioplastic film (Dou et al. 2016). Similarly, Ramakrishnan et al. synthesized bioplastic by utilizing keratin derived from chicken feathers and treated the extracted keratin suspension with various glycerol concentrations ranging from 2% to 10% (Fig. 9) (Ramakrishnan et al. 2018). The mixture of glycerol and feather was constantly stirred under magnetic stirring for 5 h at 60 °C. Subsequently, the mixture was poured in to the aluminum weighing system and then dried for 24 h at 60 °C in the oven. The bioplastic developed from 2% (wt.) glycerol and keratin mixture exhibited efficient thermal and mechanical characters. The constructed bioplastic demonstrated efficacious compatible structures without holes, cavities, and edges which was confirmed by SEM test. Thus the constructed bioplastic exhibited biodegradable properties with the potential of substituting fossil oil-dependent substances (Ramakrishnan et al. 2018). On the same line, Fernández-d'Arlas reported a new film of bioplastic made of wool keratins developed by protein suspension in a mild alkaline oxidative process that degrades disulfide bonds (Fernández-d'Arlas 2019). The original structure exhibited by the macromolecules of keratin was modified partially upon the extraction as demonstrated by the reduced ratio of α -helices to β -sheets with higher molecular mass fractions which permitted plastic properties of films and film formation. Sodium-dodecyl-sulfonic acid and glycerol were used as a plasticizer

for developing keratin films which provided various hydrophobic properties to the bioplastics. The water concentration within the keratin film depended on the humidity, being capable to absorb water content up to 35 wt. % at 80% of humidity. Also, with spectroscopy, it was analyzed that the developed bioplastic film could absorb UV light, an acknowledged application for packaging. Thermo-mechanical and thermogravimetric analysis showed more stability for keratin composites up to 200 °C with nil inherent thermal transition (Fernández-d'Arlas 2019). Likewise, Sharma et al. extracted keratin molecules to construct a bioplastic by inserting micro-crystalline cellulose acting as a nano additive agent (Sharma et al. 2018). The estimated yield for keratin from 25 g (w/w) feathers was around 80.2%. The keratin powder was mixed with 3.5% glycerol and 0.2% micro-crystalline cellulose in sodium hydroxide at 60 °C at 48 h. The estimated tensile strength and thickness for bioplastic film were 3.62 ± 0.6 MPa and 1.12×10^{-4} , respectively, thereby depicting commercial applications (Sharma et al. 2018). Furthermore, Pulidori et al. fabricated a one pot micro-wave equipped method for environment-friendly extraction of keratin from the feathers waste followed with direct electro-spinning process of extracted raw material, with any further purification processes, to procure keratin dependent bioplastics (Pulidori et al. 2021). The extracted keratin was mixed with gelatin in order to develop keratin-dependent biocompatible and biodegradable bioplastic through electron-spinning, utilizing 3-(Glycidyloxypropyl) trimethoxysilane (GPTMS) as an agent for cross-linking and thereby constructing an efficient bioplastic film with considerable commercial applications (Pulidori et al. 2021).

Keratin and Cosmetics

The application of keratin in form of keratin hydrolysates has attained considerable recognition as a potential cosmetic substrate. Keratin hydrolysates have been widely utilized as a standard constituent in nail and hair cosmetics along with skincare products (Mokrejs et al. 2017). Mokrejs et al. added keratin hydrolysates to the ointment base to understand the effect of keratin hydrolysates as a moisturizer (Mokrejs et al. 2017; Mokrejš et al. 2017). Various formulations were developed containing keratin hydrolysates (2%, 4%, 6%) on the basis of ointment base weight. The moisturizing characteristics of the keratin hydrolysates were examined by estimating a trans-epidermal loss of water, skin pH, and skin hydration. In the case of skin hydration, an ointment base with 2% keratin hydrolysates was optimal, as the elevation of 14–23% occurred in the hydration of the stratum corneum. In the case of trans-epidermal loss of water, an ointment base with 4% of keratin hydrolysates was optimal, as 26–46% of decrease was observed. Keratin hydrolysates bind the water composites at low levels of the epidermis with stratum corneum and thereby act as a humectant as well as is a good occlusive. No phase separation was observed in keratin hydrolysates/ointment base even after the storage time of 6 months, hence depicting efficient cosmetics properties (Mokrejs et al. 2017). Similarly, Barba et al. studied the topical applications for protein (keratin) hydrolysates over skin elasticity,

appearance, feel, and moisturization (Barba et al. 2008). The hydrolyzed peptides of keratin with <1000 Da molecular mass were fabricated from the wool and examined over the skin in two different formulations: an internal wool-lipids liposome solution and an aqueous suspension. Considerable differences were spotted between the treated sites and the control. The treated sites showed an elevation in elasticity and hydration due to the application of keratin peptide. Also, the formulation of keratin reinforced the integrity of the skin barrier and improved skin's water holding ability thereby demonstrating efficacy as a novel cosmetics product (Barba et al. 2008). K31 is type-1 acidic keratin, one of the major proteins present in the keratin complex of human hair and is important to maintain the tensile strength of hair (Basit et al. 2018). Basit et al. expressed the Keratin K31 gene (46 kDa) at a concentration of around 35% of all-total cell proteins in *E. coli* and protein was expressed in form of insoluble inclusion entities (Fig. 10) (Basit et al. 2018). The protein expressed was re-folded and subsequently purified via fast-performance liquid-chromatography utilizing the anion-exchange column. The re-folded and purified keratin31 protein, when used over chemically treated hair, enhanced the hair diameter up to 49%. Also, bleached hair's mechanical strength elevated almost twofold after just a single K31 treatment. K31 efficiently straightened the curly hair after a single K31 treatment for 1 h. K31 application significantly increased mechanical strength, smoothness, and diameter of damaged, harmed hair (Basit et al. 2018). Likewise, Fernandes et al. reported a peptide fragment of a type II keratin circular protein. Keratin protein

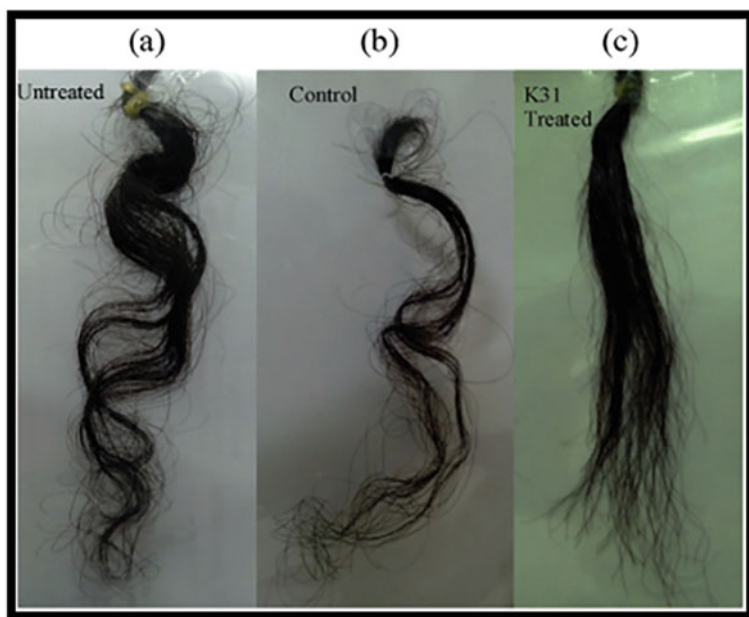


Fig. 10 (a) Untreated curly hair, (b) curly hair as control, (c) curly hair treated with K31 (Basit et al. 2018)

exhibits properties as a strengthening agent for relaxed weakened hair (Fernandes et al. 2012). The suspension of the peptide was developed both in organic solvent formulation, i.e., OF and liquid water formulation, i.e., WF to study the difference of action for both formulations in the recovery of hair. Both the formulations of peptides improved thermal and mechanical characters of damaged hair with OF peptides demonstrating the stronger effect. However, while studying the genotoxicity and cytotoxicity impact of the formulations over the skin, it was noted that OF caused irritation over the skin and possessed carcinogenic properties while WF peptides did not exhibit genotoxicity and cytotoxicity at any concentration. Therefore, WF was considered as a potential hair-strengthening agent with non-toxic effects (Fernandes et al. 2012).

Keratin and Drug Release and Tissue Engineering

Keratin exhibits potential application in tissue engineering and delivery of the drug. Various studies have been conducted to examine the potential of keratin as a biomaterial for drug delivery and tissue engineering. For instance, Srinivasan et al. fabricate a porous scaffold of keratin which was developed from a reduced suspension of keratin and showed efficient cell viability property which made it a relevant option for use in the cell seeding process (Srinivasan et al. 2010). A liquid suspension of reduced protein (keratin) was extracted by utilizing urea mixture, mercaptoethanol, water, sodium dodecyl sulfate at 60 °C from the horn meal. The molecular weight estimated for extracted keratin was ranging between 225KDa and 150KDa. The β -sheets and α -helix were confirmed by FTIR and peptide bonding for 1, 2, 3 amides were assigned as absorption bands. Thus, the developed keratin showed capability as a biomaterial for drug delivery and tissue engineering field (Srinivasan et al. 2010). On the same line, Dou et al. developed a blended film comprising of polyvinyl alcohol and feather keratin, cross-linked with dialdehyde starch for considerable drug delivery function (Dou et al. 2015). The cross-linking with dialdehyde starch improved the compatibility of polyvinyl alcohol/feather keratin mixture with a 50% decrease in melting peak and crystallinity of polyvinyl alcohol/feather keratin film. The in-total soluble weight of the constructed film inside the water at 37 °C was less than 35%, thereby depicting better film stability inside water. The release of Rhodamine-B dye tests depicted that the rate of release reduced with the increase in dialdehyde starch content. Hence, the developed film exhibited potential drug delivery parameters (Dou et al. 2015). Similarly, Cui et al. investigated the effect of transglutaminase or TGase over the characteristics of keratin from wool (Cui et al. 2013). The reported film modified with TGase was used for drug delivery and cell proliferation. TGase (30 U/g keratin) treatment at 40 °C for 18 h improved the film tensile strength from 5.18 ± 0.15 to 6.22 ± 0.11 MPa while reducing the break elongation from 83.47 ± 1.79 to $72.12 \pm 3.02\%$. Also, SEM confirmed increased compact cross-sections and a rough surface. TGase modified film showed increased molecular weights of proteins. The diclofenac release results depicted that both the films, i.e., with or without treatment of TGase caused higher

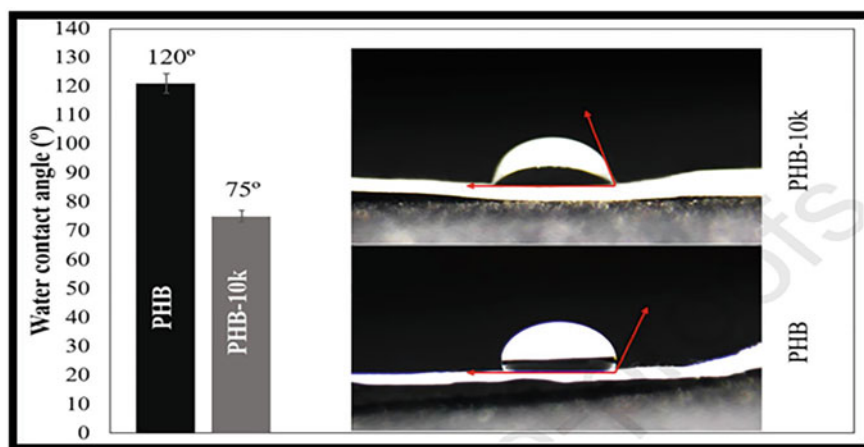


Fig. 11 PHB and PHB-10 k electrospun composite ($p < 0.05$) contact angle (Naderi et al. 2020)

release in PBS while it was relatively constant in case of gastric juice (artificial). The experiments on cell cultures indicated that the keratin films cross-linked via TGase showed better compatibility factor and thereby could be possibly utilized in tissue engineering prospects (Cui et al. 2013). Furthermore, Naderi et al. prepared new biomimetic scaffold comprising of keratin (5–15 wt. %) and poly (3-hydroxybutyrate) or PHB via electrospinning process (Fig. 11) (Naderi et al. 2020). The electrospun scaffolds exhibited 3-dimensional composites with high hydrophilicity and interconnected porosity. The presence of hydrogen bonding between 2 polymers and keratin within electrospun scaffolds was indicated by FTIR. Also, keratin presence within scaffolds increased the thermal characteristics and decreased the crystallinity of the biomimetic scaffold. The scaffolds' tensile strength elevated from 2.29Mpa to 2.92Mpa with the addition of 10(wt. %) keratin. MG-63 cell culture depicted increased proliferation and viability with the addition of keratin composites to the fabricated scaffold. Thus, significant characteristics of the electrospun PHB-10 k scaffold made it a potential substitute biomaterial for the application of bone-tissue engineering (Naderi et al. 2020). Likewise, Kakkar et al. prepared a biomimetic scaffold comprising of gelatin, keratin, and chitosan via freeze drying technique (Kakkar et al. 2014). FTIR revealed that the original structures of each utilized biopolymers were retained in the final product. Differential-Scanning-Calorimetry and TGA indicated increased thermal denaturation temperature of 200 °C–250 °C for the scaffold. Also, the keratin scaffold demonstrated compression strength of 8.5 kPa, water up-take capacity of >1700%, and tensile strength of 96 kPa in comparison to the control (collagen) with good pores interconnectivity and porosity. The cells of NIH-3 T3 fibroblast showed good cell viability for developed keratin scaffolds, hence suggesting potential biomaterial for applications in the field of tissue engineering (Kakkar et al. 2014). A mixture of hydroxycalcite comprising diclofenac and keratin for the purpose of drug release was

presented by Posati et al. (2018). The formulated hybrid film demonstrated increased thermal stability and decreased porosity, degradation, and pronounced swelling in comparison to keratin films (pure) comprising of free diclofenac. The keratin/hydroxycalcite supported fibroblast cells' growth and adhesion thereby indicating promising attributes for applications in drug release systems and tissue engineering (Posati et al. 2018).

Keratin and Wound Healing

Keratin has also demonstrated explicit application in the area of a wound dressing. Several studies have been conducted to employ keratin as a biomaterial for the dressing of wounds (Kumaran et al. 2017). For instance, Lin et al. integrated two types of naturally available polymers, keratin, and chitosan to fabricate a mobile composite membrane through UV irradiation (Lin et al. 2018). The UV-crosslinking process without any use of additional chemical reagents made the development process more relevant by utilizing reducing reactants while avoiding the use of residual noxious chemicals. The composites could possibly perform synergistic operations benefitting from keratin and chitosan as well as including better biodegradability, cell proliferation, biocompatibility, cell adhesion, and mechanical strength. The constructed novel biocomposite membrane could be employed as a wound healing biomaterial (Lin et al. 2018). On the same line, Yuan et al. reported wound healing biomaterial made from nanofibrous PHBV-Keratin composites (Yuan et al. 2012). Primarily the keratin was extracted from raw material and then chemically enhanced to attain stable keratin composite (m-keratin) following this the m-keratin was mixed with poly (hydroxybutylate-co-hydroxyvalerate) or PHBV and consequently electrospun to develop nanofibrous mats. Through 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide, or MTT assay, it was revealed that the addition of keratin increased the cell proliferation mechanism. Also, histological tests and wound-healing examinations suggested that the keratin/PHBV composite mats enhanced recovery of wound significantly in comparison to the control (PHBV) thereby, depicting as a potential candidate for wound dressing (Yuan et al. 2012). Similarly, Yao et al. constricted an efficient two-layered membrane with polyurethane acting as the outer layer while gelatin/keratin electrospun nanofibrous composites acted as the inner layer for the application of wound dressing (Yao et al. 2017). The SEM indicated that the gelatin/keratin composites exhibited uniform morphology with a bead-free system and an average diameter of fiber around 160.4 nm. Also, 3-(4,5-Dimethylthiazolyl)-2,5-diphenyltetrazolium bromide test by utilizing fibroblast cells of L929 depicted that the residual release from gelatin/keratin nanofibrous film enhanced cellular proliferation. Experiments based on cell attachment indicated that the adhered cellular bodies migrated deeper and spread better in to the gelatin/keratin mat in comparison to alone gelatin nanofibrous film. The developed bilayer membrane when treated on animals resulted in the development of more blood vessels and an enhanced reduction at the wound site within 4 days, as well as efficient wound healing at 14 days exhibiting thicker

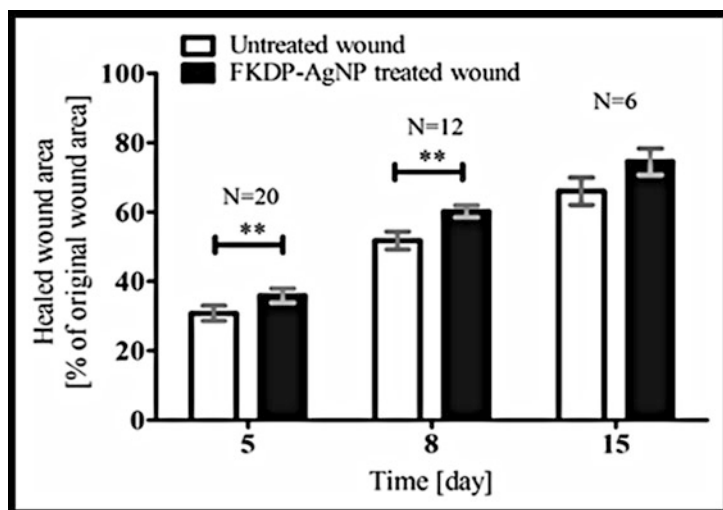


Fig. 12 Effect of FKDP-AgNP (fur-keratin-derived-powder involving silver nanoparticles) dressing on skin wound healing. Mice-numbers at day five, N = 20; day eight N = 12; day fifteen, N = 6 (Konop et al. 2020).

epidermis layer and increased production of newly developed hair follicles (Yao et al. 2017). Furthermore, Konop et al. studied the potential role of the keratin-based insoluble fur powder comprising of silver nanoparticle or AgNP on full thickness allogenic surgical skin-wound model in a diabetic mice (Fig. 12) (Konop et al. 2020). SEM revealed the presence of silver nanoparticles (single layer) over the keratin surface. Microbiological assay indicated the growth of *Staphylococcus aureus* and *Escherichia coli* to a little extent over keratin/AgNP composite. The in vitro tests revealed that the keratin/AgNP composite does not hinder the growth of fibroblast or initiate hemolysis and in vivo tests utilizing the model of diabetic mice suggested biocompatible characteristics of the developed composite. The AgNP/keratin film considerably enhanced epithelization and wound closure at days five and eight in comparison to control. Histological assays of inflammatory -response indicated that keratin/AgNP treated wounds comprised mainly of macrophages thereby favoring tissue healing and re-modeling. Hence the AgNP/keratin dressing showed considerable wound healing properties (Konop et al. 2020). A novel wound dressing having antibacterial properties was prepared from human hair and carboxymethyl cellulose or CMC with the topical clindamycin release by Sadeghi et al. (2019). Human hair was used for the extraction of keratin and various sponges were developed by altering keratin to CMC ratio which was further compared and characterized. Halloysite nano-tubes were utilized as a carrier material to manage the release of clindamycin. SEM revealed the formation of porous morphologies with inter-connected pores and uniform dispersion of clindamycin-loaded halloysite nano-tubes in the porous frame. The increase in keratin proportions with CMC

hydrogels significantly reduced rate of water vapor transmission to a relevant range and increased the water stability for CMC hydrogels in wound repair. Also, the prepared dressing efficaciously inhibited growth of *S.aureus* after 24 h. The fibroblast culturing over dressing indicated increased proliferation, spreading and cellular attachment with the elevated amount of keratin (Sadeghi et al. 2019). Likewise, Ranjbar-mohammadi et al. produced antibacterial hybrid scaffolds comprising of polyvinyl alcohol/keratin and poly (ϵ -caproactone) for the application in the field of skin regeneration (Ranjbar-mohammadi 2021). The fabricated nanofibers exhibited appropriate mechanical strength and modulus same as natural skin. The scaffold exhibited increased levels of hydrophilic properties thereby improving cell adhesion and enhanced proliferation and growth of fibroblast cell bodies. Thus, demonstrating potential dressing for wound healing (Ranjbar-mohammadi 2021).

Keratin and Industries

The keratin is also widely utilized in industries for various purposes. Research work has been conducted to study the potential of keratin in industries (Vinayak et al. 2022). For instance, Bhavsar et al. studied the characteristics of hydrolyzed keratin, generated with the help of sustainable hydrolysis method, to be utilized as the foaming reagent in the dyeing of foam for wool and cotton fabrics (Bhavsar et al. 2017). The properties such as molecular weight, blow ratio, surface suspension bubble size, and foam stability for hydrolyzed keratin in liquid suspension with dyeing auxiliaries and without dyeing auxiliaries were examined. The dyeing factors like wet pick-up were estimated to recognize the impact over color strength and dye fixation. The properties of foam dyeing were relatively compared with the standard pad-steam method and cold-pad batch for wool and cotton, respectively. The constructed hydrolyzed keratin showed decreased surface tension, better foam stability, and bubble size of 0.02–0.1 mm and 10:1 as blow ratio thereby exhibiting functions as a foaming reagent. The wool and cotton fabrics were dyed utilizing acid and reactive dyes, respectively, over a horizontal padding-mangle. Hydrolyzed keratin acted like a carrier molecule for dye and the phenomenon of dyeing depended on the particular pH of keratin, fiber, and dye solution. Foam dyeing for wool showed more color strength while cotton showed similar color strength in comparison to the conventional dyeing method. The rubbing and washing fastness of wool and cotton foam dyed material was the same as conventional dyed material. The integration of sustainable hydrolyzed keratin generation and its utilization as a biodegradable, environment-friendly foaming reagent in less add-on foam dyeing method resulted in more saving of energy and water with a minimized environment and effluent load (Bhavsar et al. 2017). Similarly, Santha Kalaikumari reported bio-utilization of waste feathers from poultry for keratinase generation and its applicable applications in the leather industry (Santha Kalaikumari et al. 2019). The keratinase was extracted from *Bacillus paralicheniformis*-MKU3, a keratinolytic bacterium from waste feather. Statistical assays were performed to increase the performance of extracted keratinase which subsequently improved

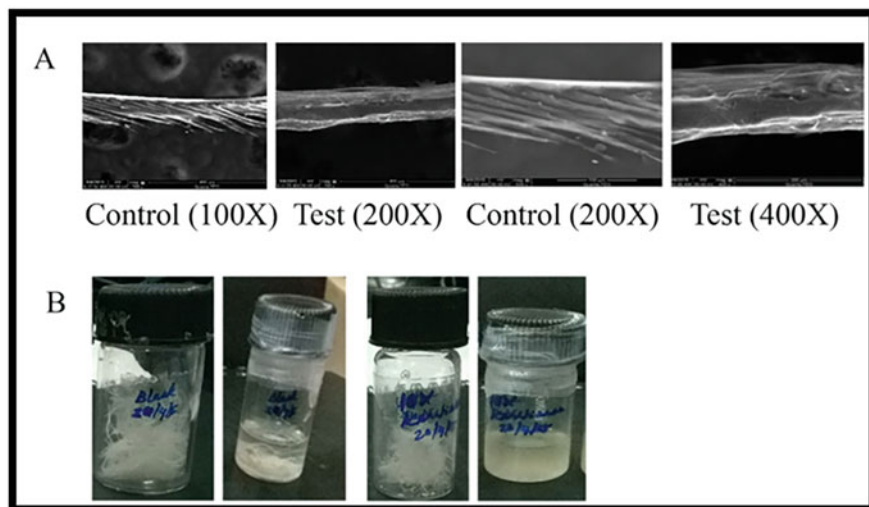


Fig. 13 Chicken feather hydrolysate, Blank 0 h (Feather before water addition), Blank 24 h Feather after 24 h in water (Control), Test 0 h (Feather before Enzyme addition, Test 24 h (Feather after 24 h of the enzymatic treatment) (Santha Kalaikumari et al. 2019)

enzyme activity by sixfold with a maximum yield of 1872.5 U/ml and a specified performance of 1143.19 U/mg in 72 h. The extracted keratinase hydrolyzed 90% of the waste feather and caused 100% dehairing of sheepskin which was confirmed by histochemical assays and SEM (Fig. 13). The processed crust of leather demonstrated efficient histo-chemistry and physical strength (Santha Kalaikumari et al. 2019). On the same line, Zhang et al. isolated keratinase generating bacterium from the duck ranch-soil and classified it as an *Acinetobacter* species R-1 on the basis of 16S rDNA sequence and biochemical properties (Zhang et al. 2016). The extracted keratinase exhibited low activity of collagenase and high activity of keratinase. Also, the keratinase enzyme was purified by electrophoretic homogeneity suggesting 25 kDa ad molecular weight, 2.68 folds purification with 6.69% recovery. The significant keratinase activity was indicated a pH 11, temperature 50 °C, and elemental presence of Li^+ , Ca^{2+} , and Na^+ while decreased in presence of EDTA thereby characterized as Metallo-keratinase. The wool surface was considerably modified and the goat skin was thoroughly depilated by the extracted keratinase, hence suggesting as a potential biomaterial for use in textile and leather industries (Zhang et al. 2016). Furthermore, Niculescu et al. fabricated a biocomposite derived from keratin and collagen retrieved as a by-product from the leather industry (Niculescu et al. 2019). The keratin and collagen were extracted from the by-products of sheep wool and bovine leather and further cross-linked with suitable chemical reagents for decreased environmental impact. The developed biocomposites depicted properties like controlled nitrogen release, pelliculogenic nature, and rape seedlings nutrition, thereby suggesting it as a potential candidate for

agricultural applications while the properties like leather finishing indicated the utilization of developed biocomposite in industries. Moreover, the integration of odorous factors with optimized release demonstrated the eco-friendly property of biocomposite (Niculescu et al. 2019). Hussain et al. fabricated a sponge from the keratin waste and tested it to eliminate chromium traces from wastewater (Hussain et al. 2020). The desorption mechanism revealed that at 10 pH or above, the developed sponge could be re-used with feasible chromium recovery. The continuous flow method and sorption process were carried out to examine the industrial relevance of the sponge. The keratin sponge could be utilized to manage the industrial treatment method as a fix-bed column for the continuous elevation of chromium ions, the elimination results depicted that the keratin sponge could attain 91% of elimination efficiency with 270.27 mg/g as adsorption capacity (Hussain et al. 2020) (Table 1).

Advancement in Keratin-Derived Three-Dimensional Structures

The bio-scaffold printing via three-dimensional method is an acknowledged technique. Three-dimensional keratin scaffolds can be customized and quick to develop thereby suggesting patient-specific medical approach. Various advancements have been witnessed in the development of 3-D keratin scaffolds. For instance, biomaterial based on keratin-lignin biocomposite indicated considerable potential to develop fourth dimensional response following three-dimensional printing (Grigsby et al. 2020). Moreover, digital-light-process/three-dimensional printing-based bioink (four-arm-polyethylene-glycol-acrylate/silk fibroin) printed excellent keratin film and hence depicted a quick printing technique with optimized fabrication of thin large-sized hydrogels multilayers of several designs (Kwak et al. 2019). The fibers generated from the combined composite of poly lactic acid/chitosan/keratin exhibited applications in tissue engineering with applicability in bone regeneration (Rojas-Martínez et al. 2020). The keratin hydrogels composed of hydroquinone-SPS-riboflavin (inhibitor-catalyst-initiator), photo-sensitive suspension demonstrated efficient cell adhesion and migration properties for effective use in the fields of regenerative medicine and tissue engineering (Placone et al. 2017). Therefore, three-dimensional printed keratin scaffolds have undergone various developments with still studies under process for clinical and large-scale applications in various fields, specifically tissue engineering and regenerative medicines.

Challenges and Future Prospects

The main challenge which lies in keratin application is the extraction method. The till-date used extraction methods are non-biodegradable, laborious, and expensive. Though sustainable methods have been proposed but still large-scale employment is under progress. Also, the regular effective use of keratin films in the field of bioplastics, cosmetics, drug delivery, and tissue engineering, wound healing and

Table 1 Discussed applications of keratin

Application	Keratin employed	Substrate	Eco-friendly property	Analyzed parameters/outcomes	Use	References
Bioplastics	Acid precipitation and alkaline: The hydrolyzed keratin	Glycerol concentrations via a thermoplastic method	Yes	Water addition during hot-pressing and glycerol concentration	Agriculture, packaging	Dou et al. (2016)
Bioplastics	Alkaline extracted keratin	The mixture of glycerol and feather	Yes	2% (wt.) glycerol and keratin mixture	Substitute for fossil oil-dependent substances	Ramakrishnan et al. (2018)
Bioplastics	Keratin derived from a mild alkaline oxidative process	Sodium-dodecyl-sulfonic acid and glycerol	Yes	Water content up to 35 wt. % at 80% of humidity, thermal stability(50-200oC)	Regenerative medicine, coatings	Fernández-d'Arlas (2019)
Bioplastics	Alkaline extracted keratin	3.5% glycerol and 0.2% micro-crystalline cellulose in sodium hydroxide	Yes	Tensile strength and thickness for bioplastic film were 3.62 ± 0.6 MPa and 1.12×10^{-4} , respectively	Industries	Sharma et al. (2018)
Bioplastics	Keratin derived from one pot micro-wave	Keratin/gelatin/GPTMS	Yes	GPTMS/keratin concentrations	Purification membranes, bio-packaging	Pulidori et al. (2021)
Cosmetics	Keratin hydrolysates	Ointment base	Yes	2%, 4% of keratin hydrolysates	Skin moisturizer	Mokrejs et al. (2017)
Cosmetics	Keratin hydrolysates	An internal wool-lipids liposome solution and an aqueous suspension	Yes	Internal wool-lipids liposome	Skin moisturizer	Barba et al. (2008)
Cosmetics	Keratin insoluble inclusion entities	K31 gene	Yes	Straightened the curly hair after a single K31 treatment for 1 h and hair diameter up to 49%	Hair treatment	Basit et al. (2018)

Cosmetics	Reported peptide fragment of a type II keratin circular protein.	Organic solvent formulation i.e. OF and liquid water formulation i.e. WF	Yes	WF peptides did not exhibit genotoxicity and cytotoxicity at any concentration.	Relaxed weakened hair	Fernandes et al. (2012)
Drug release and tissue engineering	Reduced suspension of keratin	The porous scaffold of keratin	Yes	Keratin molecular mass 225KDa to 150KDa with the presence of β -sheets and α -helix	Biomaterial for drug delivery and tissue engineering field	Srinivasan et al. (2010)
Drug release	Feather keratin	Polyvinyl alcohol/feather keratin/dialdehyde starch	Yes	Conc. Of dialdehyde starch	Drug delivery parameters	Dou et al. (2015)
Tissue engineering	Wool keratin	TGase	Yes	TGase (30 U/g keratin) treatment at 40 °C for 18 h improved the film tensile while reducing the break elongation	Drug delivery and cell proliferation.	Cui et al. (2013)
Tissue engineering	Keratin powder	PHB/keratin	Yes	Keratin concentration	Bone - tissue engineering	Naderi et al. (2020)
Tissue engineering	Keratin precipitation via SDS	Gelatin, keratin and chitosan	Yes	The scaffold compression strength of 8.5 kPa, water up-take capacity of > 1700%, and tensile strength of 96 kPa	Biomaterial for tissue engineering	Kakkar et al. (2014)
Drug delivery	Sulfitolysis extracted keratin	The mixture of hydrothermalite comprising diclofenac and keratin	Yes	Increased thermal stability and decreased porosity, degradation, and pronounced swelling	Drug release platform	Posati et al. (2018)
Wound healing	Reduced keratin	Keratin/chitosan/UV-crosslinking	Yes	Better biodegradability, cell proliferation, biocompatibility, cell adhesion, and mechanical strength	Wound healing bio-material	Lin et al. (2018)

(continued)

Table 1 (continued)

Application	Keratin employed	Substrate	Eco-friendly property	Analyzed parameters/ outcomes	Use	References
Wound healing	m-keratin	PHBV-keratin composites	Yes	Keratin concentration	Wound healing biomaterial	Yuan et al. (2012)
Wound healing	Acid precipitation of human hair keratin	Polyurethane/gelatin/keratin	Yes	Reduction at the wound site within 4 days, as well as efficient wound healing at 14 days	Wound dressing bio-material	Yao et al. (2017)
Wound healing	Keratin-based insoluble fur powder	Keratin/AgNP	Yes	The AgNP/keratin film considerably enhanced epithelization and wound closure at day five and eight	Wound dressing bio-material	Konop et al. (2020)
Wound healing	Reduced keratin precipitated from human hair	CMC/keratin	Yes	Appropriate keratin concentration	Wound dressing bio-material	Sadeghi et al. (2019)
Wound healing	SDS precipitate wool keratin	Polyvinyl alcohol/keratin and poly (ε-caproactone)	Yes	50:50 ratio of keratin and polyvinyl alcohol	Skin regeneration	Ranjbar-mohammadi (2021)

Industries	Hydrolyzed keratin	Dyeing auxiliaries	Yes	The pH of keratin, fiber, and dye solution	Foaming reagent in the dyeing of foam for wool and cotton fabrics	Bhavsar et al. (2017)
Industries	The keratinase from bacillus paralichemiformis-MKU3	Keratinase	Yes	The extracted keratinase hydrolyzed 90% of the waste feather and caused 100% dehairing of sheepskin	Leather industry	Santha Kalaikumari et al. (2019)
Industries	Keratinase from Acinetobacter species R-1	Keratinase	Yes	The keratinase activity was indicated a pH 11, temperature 50 °C, and elemental presence of Li ⁺ , Ca ²⁺ , and Na ⁺	Textile and leather industries	Zhang et al. (2016)
Industries	Reduced keratin as a by-product from sheep wool	Keratin/collagen	Yes	Keratin/collagen increase biomass by 38% for rapeseed	Agricultural applications and leather finishing	Niculescu et al. (2019)
Industries	Reduced keratin a waste from the leather industry	Keratin/glutaraldehyde/acetone/liquid nitrogen	Yes	10 pH or above, the developed sponge could be re-used with feasible chromium recovery.	Wastewater treatment	Hussain et al. (2020)

industries is under improvement rate. Thereby, specific studies must be conducted so as to develop sustainable, cost-effective, and efficient keratin extraction methods and keratin bio-based films to enhance the versatility of the biomaterial in various sectors, fields, and areas of life.

Conclusion

Keratin is a promising naturally derived protein possessing notable properties which increase its commercial significance. The degradation and extraction of keratin is the primary and important prospect for the employment of keratin as a biomaterial. The discussed microbes (RCS6 a yeast strain, *Fusarium* species 1A, *Bacillus* species 8A6, *B. subtilis* 8) and green solvent (NMMO) suggested efficient and eco-friendly degradation of keratin which preferably could be used for large-scale keratin degradation with a considerable reduction of keratin waste in the environment. Further, the mentioned sustainable solvents, urea-sodium acetate, L-cysteine and lactic acid, hydrated ionic solution, Choline chloride and oxalic acid, and ultrafiltration process demonstrated effective, eco-friendly, and better regeneration of keratin with good keratin yield from the keratinous waste. Moreover, developed keratin films exhibited explicit properties such as cell attachment, biodegradability, cell proliferation, biocompatibility, mechanical strength, increased thermal stability and decreased porosity, degradation, pronounced swelling, better skin moisturizing ability, toughness, etc. which remarkably increase the commercial application of keratin in several sectors. However, sustainable extraction of keratin should be promoted to convert and efficaciously use the keratin waste for developing better keratin films with considerable applications, thereby promoting sustainable development which is the need of the hour.

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C. S. Julie Chandra, Sreesha Sasi, and T. K. Bindu Sharmila

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Abstract

Gelatin, a well-known biocompatible polymer, is a derivative of collagen obtained by the heat denaturation of collagen. The properties of gelatin vary depending on the sources and extraction techniques. Gelatin is remarkably known for its special gel-forming ability, which makes it a suitable material for exploring the fundamental functional features in colloid studies. Gelatin is widely explored by the scientific community because of its unique characteristics and is highly demanded by various industries such as food, pharmaceutical, cosmetic, photographic, etc. This chapter focuses on the various sources, extraction, and properties of gelatin and provide an insight into gelatin in recent applications.

C. S. Julie Chandra (✉) · S. Sasi · T. K. Bindu Sharmila
Department of Chemistry, Maharaja's College, Ernakulam, India
e-mail: juliechandra@maharajas.ac.in

Keywords

Gelatin · Collagen · Bloom index · Fish gelatin · Gelatin hydrolyses · Biomedical applications · Food applications · Photography

Introduction

The word “gelatin” is derived from the Latin word “gelata.” This refers to the substance’s ability to generate water gels, which is its most unique property (Haug and Draget 2009). One of the most widely used proteins, gelatin, is produced by hydrolyzing collagen, which makes up the majority of the natural components of skin, bones, and connective tissues (Rigueto et al. 2021). It can also be derived through fish and insects in addition to the skin and bones of land animals. It is well known that gelatin has biocompatible and biodegradable properties similar to that of collagen. Gelatin is a fairly common protein that is made by denaturing collagen (Ki et al. 2005). Although collagen and gelatin have approximately the same relative quantities as well as sequencing of the various amino acids, they differ greatly in terms of physical properties.

Collagen is the main component of all white fibrous connective tissues that are present in animal bodies including cartilage, skin, the translucent sheaths that enclose muscles and muscle fibers, as well as ossein. Gelatin is easily dissolved in water, when heated to temperatures exceeding the denaturation temperature of natural collagen, however, collagen is insoluble in water. Under the same circumstances, collagen just contracts and loses its capacity to store water (Gómez-Guillén et al. 2009). At the same time, gelatin displays unique characteristics that collagen either lacks or just slightly develops (Ockerman and Hansen 1999). Due to the presence of both single and double open chains, gelatin has hydrophilic nature (Wittaya 2012). Gelatin expands when submerged in a liquid because it absorbs moisture. The inflated particles melt as the liquid is heated, creating a sol (fluid colloidal system) that thickens as it cools and solidifies to form a gel. At higher temperatures, the gel state can be reversed into a sol state, and the sol can be converted back to a gel through cooling. Temperature, protein, and sugar concentration all influence setting time and tenderness.

In general, depending on how collagen is pretreated, two types of gelatin, viz. A- and B-type gelatin, can be synthesized. Acid-treated gelatin is type A and its isoelectric point is at pH 6–9. The Pig skin’s collagen has a lower degree of covalent cross-linking which on acid treatment can form type A gelatin. The alkaline-treated collagen can be named as B-type gelatin, which has an isoelectric point at pH 5 (Al-Nimry et al. 2021). Type B gelatin can be synthesized from the collagen present in cow skins. Aramwit and Srichana claim that type B gelatin nanoparticles have a higher degree of cross-linking than type A gelatin nanoparticles, which results in a slower rate of disintegration (Aramwit et al. 2015). B-type gelatin nanoparticles have a range of physicochemical characteristics that would allow them to release and interact with different substances that have an impact on these groups. Owing to the

presence of the functional amino acid group, gelatin exhibits an overall amphoteric behavior, having both acidic and basic activities (Su and Wang 2015).

Due to its low cost, abundance, availability, film-forming capacity, flexibility, odorlessness, and capacity to serve as a good carrier, gelatin has come to be recognized as a biopolymer material (Chanchal et al. 2014). The fact that gelatin molecules include both carboxyl and amine functional groups is the first feature of gelatin that all biopolymers share. The second characteristic of gelatin that sets it apart from other polymers is its capability to produce a precise type of triple-stranded helical structure (this structure is formed in solutions at low temperatures). The types of amino acids present and the strength of the gelatin in the solution are only a few of the variables that affect how quickly a helical structure forms. The distinctive interaction of gelatin with water, which is dissimilar from synthetic hydrophilic polymers, is the third feature of gelatin as a biopolymer. This peculiarity controls the solid-state structural and physicomachanical characteristics of gelatin (Lin et al. 2018).

As one of the most adaptable biopolymers, gelatin has an extensive range of uses in food, sweets, medical items, cosmetics, and technical goods. Even before this, gelatin was utilized in cuisine, and scientists have been researching and studying it at least since the early twentieth century. Gelatin is mostly utilized in the solid state, with the exception of the food industry (Karim and Bhat 2008).

However, because to their hydrophilic properties, gelatin-based polymers have some limits in high-moisture environments and perform poorly mechanically. Reinforcing additional materials into the polymeric matrix as composites or nano-fillers is one way to get around these constraints.

Sources of Gelatin

Gelatin is extracted from sources of collagen which are mainly bovine, porcine, fish, poultry, camels, and even amphibians such as frog and salamander (Abedinia et al. 2017; Ahmed et al. 2020; Al-Hassan 2020; Chakka et al. 2017; Jin et al. 2019; Karnjanapratum et al. 2017; Lv et al. 2019; Nik Muhammad et al. 2018; Sultana et al. 2018). Of these, hide from hog is the prominent precursor in the manufacture of gelatin. Pork skin accounts for around 45% of all gelatin produced globally and bovine hides at around 30%. Approximately 23% of gelatin is made from the bones of cows and pigs. Fish and chicken are additional sources; however, they only contribute 1.5% to the annual global gelatin production (Karim and Bhat 2009).

Despite the fact that gelatin has a broad spectrum of uses, some consumers are apprehensive about its consumption and usage. This is primarily attributed to religious sentiments because Hindus are concerned about using bovine meat and bones, while Islam and Judaism condemn the usage of pork-related products (Karim and Bhat 2008).

Concerns regarding probable virus and prion contamination, such as the bovine spongiform encephalopathy (BSE) prion, which caused the human Creutzfeldt-Jakob

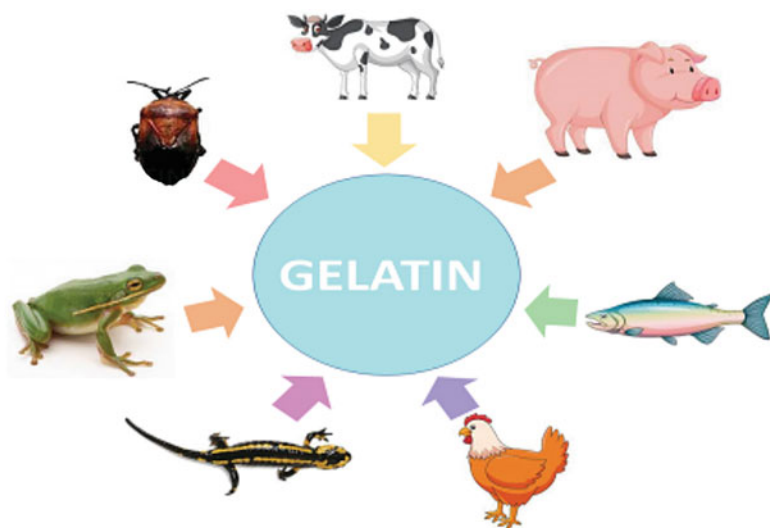


Fig. 1 Sources of gelatin

disease (CJD) in the 1980s, are also mentioned in addition to religious considerations (Karim and Bhat 2008).

According to latest researches, fish scales and skin could serve as an alternate resource of gelatin. Since fish skin/scales gelatins have lower gel strengths and melting temperatures than mammalian gelatins, they might be a superior option for some applications. For some food systems, these qualities may be desired since they make it easier for flavors to release, which improves the sensory qualities (Karim and Bhat 2009). Another alternative source of gelatin is insects. Gelatins extracted from *Agonoscelis pubescens* (sorghum bug) and *Aspongopus viduatus* (watermelon bug) were found to be suitable for human consumption and were similar to mammalian gelatins in structure, properties, and uses (Mariod et al. 2011). The various sources of gelatin are depicted in Fig. 1.

Extraction of Gelatin

Hydrolysis of collagen is the most critical and basic step in the course of extraction of gelatin (Baziwane and He 2003). There are five fundamental steps involved in the transformation of raw materials into gelatin: washing, extraction, purification, concentration, and drying (Johnston-Banks 1990). Optimizing the pH, temperature, and extraction time is pivotal since the extraction procedure is intended to produce the highest yield along with the most useful characteristics. Water extractions are carried out thrice or four times at progressively higher temperatures to obtain gelatin. Typical temperatures from 55–90 °C are used for the extractions. Collagen can be hydrolyzed into gelatin by essentially two different methods. One is the acid method,

which mostly uses raw materials like pig skin, sea fish hide, and occasionally ossein. Here, collagen is heated gradually in order to denature and solubilize the collagen into type A gelatin (Reich et al. 1962).

The second method is the alkali process, which is mostly applied to cattle skin (Cole and Roberts 1996). Prior to extraction, collagen is exposed to processing with sodium hydroxide that swiftly cleaves the side chains of asparagine and glutamine, giving gelatin its conventional isoionic point between four and five. This gives type B gelatin (Veis 1964).

To eliminate the suspended insoluble materials such as lipids or unhydrolyzed collagen fibers, the extracted solution of gelatin is subsequently passed filtered using a diatomaceous earth filter. After that, additional refining is done using ion exchange or ultrafiltration columns, which removes inorganic salts and brings the pH level to 5.0–5.8, as required for commercial gelatin. Dehydration (mostly by spray drying), sterilization, and drying make up the last step. To reduce the loss of physicochemical properties of the resulting gelatin, these three-unit operations are carried out as rapidly as possible. The finished product is subsequently put through a series of qualitative analysis tests, including ones for composition, viscosity, bloom strength, and microbiological testing.

Chemical Composition and Structure of Gelatin

Gelatin consists of 18 different amino acid residues having a fundamental repeating unit Gly-Pro-*x*- or Gly-*x*-Hyp, where *x* is a basic or an acidic amino acid. Glycine, proline, and hydroxyproline make up about 56% of the chemical composition of gelatin and glutamic acid, alanine, arginine, and aspartic acid making up the remaining 44% roughly. Glycine or alanine account for approximately 33–50% of amino acid residues. Glycine is the most prevalent N- terminal amino acid in alkali-processed gelatin, whereas alanine is more dominant in the acid-processed one. A 25% of the amino acid residues are proline or hydroxyproline, and nearly 25% is basic or acidic. The lack of the essential amino acid tryptophan and aromatic acid residue in gelatin has been indicated. Figure 2 depicts a typical gelatin structure (Bender et al. 1953; Charley 1992; Cole 2000; Djagny et al. 2001; Loofbourow et al. 1949; Sultana et al. 2018).

Gelatin is a heterogeneous mixture of various polypeptide chains having 300–4000 amino acids. There are α -chains, which are single chains; β -chains, which are two α -chains having covalent bonding between them; and γ -chains which consist of three α -chains having covalent linkage among them and has a molecular mass of around 15–400 kD (Alipal et al. 2021; Bagal-Kestwal et al. 2019). An approximate percentage of the various amino acids in bovine gelatin is given in Fig. 3 (Aykın-Dinçer et al. 2017). Gelatin contains 25.2% O, 6.8% H, 50.5% C, and 17% N (Hanani et al. 2014).

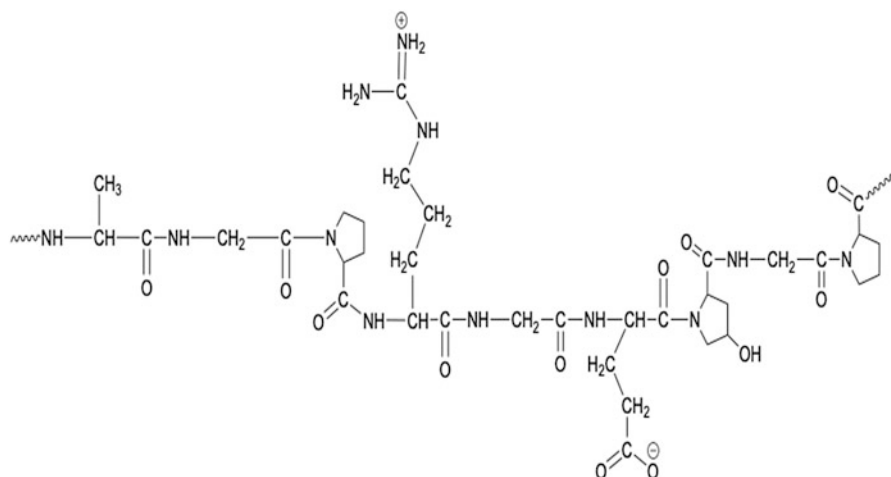


Fig. 2 Basic chemical structure of gelatin

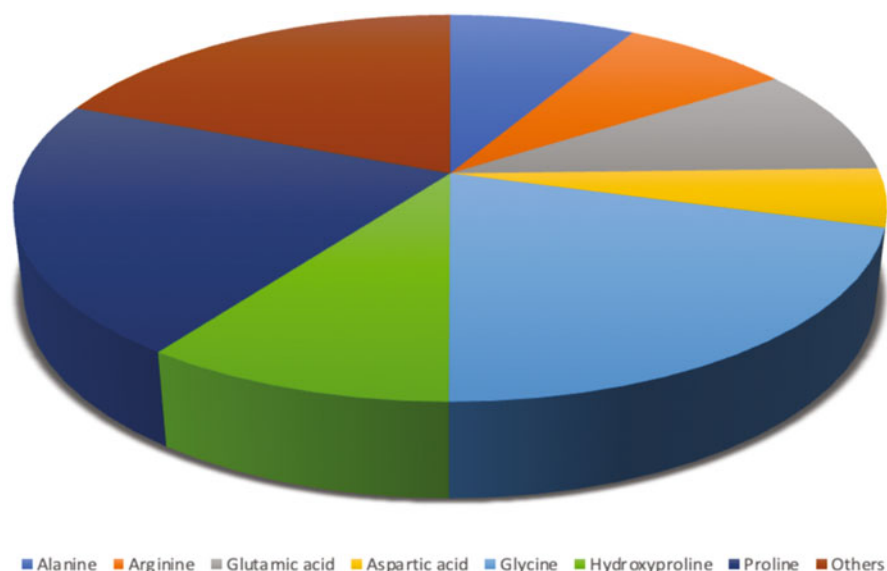


Fig. 3 Approximate percentage of various amino acids in bovine gelatin

Properties of Gelatin

Gelatin, a water-soluble protein, is a hydrolyzed product of collagen. It is generally an odorless, tasteless, translucent, and fragile solid. It appears pale yellow to amber in color (Anding 1951). It is a high molecular weight polypeptide ranging from 15–40 kDa. The molecular weight varies depending on the processing conditions.

The moisture content of gelatin is found to be 8–13%. It has a specific gravity of 1.3–1.4. Gelatin forms swollen particles when soaked in water and dissolves to form solution on warming. Behavior of gelatin solutions is influenced by temperature, pH, ash content, method of manufacture, thermal history, and concentration (Gelatin Handbook 2012). The solubility of gelatin depends on the nature and polarity of solvents. Gelatin dissolves in hot water, and is soluble in aqueous solutions of polyhydric alcohols such as glycerol, propylene glycol, etc. In contrast to less polar organic solvents like benzene, acetone, primary alcohols, and dimethylformamide, it is dispersible in organic solvents with high polarity including acetic acid, trifluoroethanol, and formamide (Gelatin Handbook 2012).

Fish and poultry gelatin have drawn a lot of interest in the past few years. Mammalian and fish gelatins both share similar functional properties. It comes from swim bladder, scales, fins, bones, or skin. Giménez et al. found that adding more gelatin hydrolysates to squid gelatin films decreased pierce force and enhanced water vapor permeability (WVP) (Giménez et al. 2009). According to Haug et al., the main imino acid differential between fish gelatin and its mammalian analogues may be the cause of the lower gelling and melting temperatures as well as the low gel modulus (Haug et al. 2004). In fact, the imino acid level in gelatin varies with the origin of fish. Gelatin derived from warm water fishes like tilapia and bigeye tuna have lower imino acid contents than those derived from cold water (such as halibut and cod) (Eastoe and Leach 1977).

The functional properties like gel strength (bloom index), viscosity, setting behavior, and melting point are related to their molecular weight distribution, amino acid combination, etc. Gelatin having higher levels of amino acids tends to show higher gel strength (in units of bloom) and melting point. Gel strength, also referred to as bloom value, is a measurement of the stiffness and strength of gelatin that takes into account the average molecular weight of its constituents (Haug and Draget 2009). It indicates the resilience of a jelly sample of known strength tested under standard conditions. It typically ranges from 30–300 bloom (below 150 is regarded as a low bloom, between 150 and 220 as a medium bloom, and between 220 and 300 as a high bloom). Greater gelatin strength is indicated by higher bloom values. Gel strength and viscosity are the most useful properties of gelatin which are gradually weakened on prolonged heating in solution above 40 °C. Gel strength depends on the α and β chain components in gelatin. Applications of gelatin is determined by the gel strength. For instance, the confectionary industry uses mainly type B gelatine having gel strengths in the range 125–250. Gelatin with a lower gel strength of 70–90 (type A gelatine) is used for the fining of wine and juice (Wittich 2005). According to Sarbon et.al, gelatin made from chicken skin exhibited more gel strength (355 g) and stability than beef gelatin (259 g) (Sarbon et al. 2013). The amino acid compositions of gelatin vary with the species from which it is derived, as previously described, especially with regard to the amino acids glycine (Gly), proline (Pro), and hydroxyproline (Hyp). With regard to its gelling capabilities, proline and hydroxyproline content of gelatin is mainly crucial. The molecular weight distribution and amino acid composition are the primary elements that affect the physical and structural characteristics of gelatin, as well as the mechanical and

barrier properties of the resultant films. Gelatin films are translucent and have good gas barrier characteristics.

The nature of gelatin, concentration, duration, and temperature have a significant effect on the viscosity of gelatin. The intrinsic viscosity of gelatin processed by acid (type A) and alkali (type B) appears to be slightly different, however there is no discernible difference in their melting points. The setting point of a gelatin solution is determined by its thermal and mechanical conditions. In contrast to brisk cooling, delayed cooling of the solution results in higher setting temperatures. Setting temperatures of gelatin can be hindered or delayed by mechanical agitation (Simon et al. 2003).

Gelatin is a material that is created in powdered or granulated form and has no flavor or odor. It can be utilized as a fastening, gelling, foaming, emulsifying, and film-forming agent. Due to its hygroscopic nature, gelatin will collect moisture based on the humid conditions at which it is dried and kept (Karim and Bhat 2008).

Gelatin is amphoteric in nature, capable of behaving either as an acid or as a base. Isotonic point of gelation ranges from pH 5.0–9.0 depending on the source and manufacturing techniques. Acid-processed type A gelatin has isoionic point ranges from 7–9, and that of type B it is between 4.8 and 5.1. The isoionic point, together with dispersion and gelation, are the most crucial factors in governing how gelatin is used, particularly in the food industry (Kobayashi 1996).

Gelation property of gelatin is not clearly explained until today. Several mechanisms were suggested. The presence of both acidic and basic functional group in the gelatin molecules gives gelatin specific features. It can form triple-stranded helical structure. Another peculiarity of gelatin is its specific interaction with water molecules which governs the structural and physicommechanical properties of gelatin (Kozlov and Burdygina 1983).

Gelatin can dissolve in water at about 35–45 °C and forms gel on slow cooling (Bagal-Kestwal et al. 2019). It is suggested that a several gelatin molecules unite to form crystallites. These crystallites form a three-dimensional network that can immobilize the liquid, converting to a gel. As reported by Ferry, hydrogen bonds and van der Waals forces help to bind the gelatin molecules to form a gel whereas Bello and Vinograd proposed peptide linkage (Bello et al. 1962). The properties of gelatin gels depend on the speed of cooling and degree of acidity. The proper orientation of gelatin molecules on gelation can be achieved during slow cooling. The gel setting time can be prolonged and the liquifying temperature can be lowered as acidity increases. A very low liquifying temperature of about 28 °C is shown by majority of edible gelatin gels (Kobayashi 1996). On heating the gel, it melts and turns back into solution. Thus the sol-gel conversion is reversible and is recognized as thermoreversible gel (Bagal-Kestwal et al. 2019). Gelatin solution can have film-forming ability, when poured evenly on a smooth surface and dried. This property can be made useful in the manufacture of soft and hard capsules and microencapsulation (Bagal-Kestwal et al. 2019). Additionally, gelatin has been said to have antioxidant properties and antibacterial properties. Gelatin was one of the earliest materials employed to transport bioactive components (Gennadios et al. 1994). The ability of gelatin to interact with other substances, especially macromolecules, is of

great implication for explicating its applications in various fields, particularly in food and pharmaceutical industry. The major part of many investigations focuses on the compatibility of gelatin with food components (Djagny et al. 2001).

Applications of Gelatin

Gelatin is a multipurpose natural macromolecule that finds an extensive spectrum of applications such as in food industry, biomedical, photography, cosmetics, environmental applications, and various other applications. An illustration of the various applications of gelatin is given in Fig. 4.

Food Applications of Gelatin

Gelatin is a partly decayed by-product of collagen found in animal connective tissue. It is a white or yellowish, transparent, glossy solid. Due to its functional qualities, such as its capacity to bind water, produce gels, operate as a water vapor barrier, form films, create foam, and have an emulsification propensity, gelatin is generally utilized in the diet, pharmaceutical, photographic, and makeups sectors (Bailey and Paul 1998). Although the gelatin exhibits excellent water absorption and swelling properties, it has poor mechanical resistance and is permeable to water vapor molecules (Xiaoqing et al. 2010). As a result, gelatin's usage as a wrapping material is restricted due to its poor barrier performance (Sazedul et al. 2011). By

Fig. 4 Applications of gelatin. (Images sourced from Internet)



combining gelatin with other useful components and active substances, this can be improved (Rhim et al. 2013).

The creation of safe and sanitary edible coatings and films is currently gaining popularity in food preservation packaging industry, taking into account the influence food safety has on consumer health. Due to their high biocompatibility and consequently increased biological and technological utility, biopolymers are frequently utilized in the food sector to create films and coats.

By causing a thorough or limited flocculation or sedimentation of liquified substances or suspended fragments for turbidity, gelatin is used to achieve the clarity of a solution and the durability of this clarity. Gelatin should only be used for these purposes in drinks and foods that contain tannins. The production of marshmallows, that is a staple of the American diet, also uses gelatin. Gelatin is frequently used in bakery goods as a stabilizing agent or setting agent in pies, breadstuffs, and cakes. Considerable amounts of edible gelatin are used in the meat business, one of the main sources of food, most notably in the production of canned hams, meat jellies, meat loaves, sausages, and cheese.

Due to their significant role in the food sector, the utilization of gelatin in frozen fruit pulp desserts and turkey goods as well as the manufacture of jellied items must be mentioned (Djagny et al. 2001). Due to the excessive protein and amino acid content of gelatin, it is frequently employed as a dietary ingredient and in nutritious foods. Through the use of processing processes such defatting, pulverization, freezing, and drying, edible gelatin is created from animal skin and bone (Fu et al. 2000).

Gelatin has several uses in the food sector due to its distinctive hydrocolloidal structure. These can be categorized into four main groups, including milk products (to provide stabilization), nonveg foodstuffs (to provide water binding), confectionery (primarily for offering texture and lather stabilization), and jelly confectionaries (to provide creaminess, fat lessening, and to make physical sensation in the mouth). Hydrolyzed gelatin applications can also be found in these categories (Nishimoto et al. 2005; Karim and Bhat 2009).

In general, gelatin is employed in cookery as a yoghurt thickener, liquid refreshments and juice clarifier, and dessert thickener. Another usage is as a stabilizer in ice cream and cheese, as well as in food stuffs and fruit salads. Other uses comprise fruit toppings for pastries, quick pottages and soups, and appetizing films for confectionary items (Karim and Bhat 2008). Additionally, gelatin is used to coat beef products to prevent color deterioration; this coating is equally effective when stored in the light or dark (Antoniewski et al. 2007). To contain the liquids lost during cooking and to act as an excellent heat transfer medium, gelatin is utilized in preserved nonveg food stuffs such as red meat, breads, Vienna sausages, and dried, tinned pigs. In addition, gelatin is utilized in creamed meats and gelled goods at quantities extending from 0.5–3 well-designed qualities for use in food applications, gelatin is absolutely extraordinary. Table 1 below lists some of the functional aspects of gelatin in diet (Mariod and Adam 2013; Turner 1988).

Gelatin is compatible with a number of biodegradable polymers that are frequently employed in appetizing coats and films, which are frequently utilized in fresh items and enhance their preservation value. As a result of its special

Table 1 Functional aspects of gelatin

Application	Gelatin function
Gelling agent	Cold meat, sweetmeat, pate, porridge, and gelled desserts
Creaming agent	Chiffons, mousses, soufflés, nougats, marshmallows, and whipped cream
As lyophilic colloid for protection	Ice cream, frosting, and other frozen sweets
Connecting agent	Rolls of meat, canned meats, sweets, cheeses, and dairy goods
Clear up agent	Vinegar, fruit juices, beer, and wine
Binders	Covering for meats and fruits
Hardener	Dairy goods, bouillon, gravies, sauces, soups, puddings, jellies, and powdered drink mixes
Processing aid	Microencapsulation of vitamins, oils, tastes, and colors
Compounding	Cream-based sauces, soups, meat pastes, confections, and dairy products
Preserver	Ice cream, chocolate milk, yoghurt, cream fillings, and cream cheese
Glueying agent	Nonpareils, coconut, and other ingredients to sweets; to bind layers of sweets together; to adhere frostings to baked goods; and to adhere seasonings to meat preparations

antibacterial and antioxidant qualities, it is a useful material for keeping fresh food. Food packaging is severely constrained owing to the high water-soluble and viscous properties of gelatin, which are both strongly influenced by atmospheric humidity and weather. It is challenging to produce the perfect fresh appearance when conserving crops and marine items because the surface structure of gelatin film is damaged when soluble matter and liquid are lost.

In order to overcome the fundamental disadvantages of virgin gelatin film, which is readily soluble in water, scientists generally combine gelatin with a common biopolymer and add certain naturally occurring active components to make reformed edible composite films and coatings. The reformed synthesized films have advantageous biological (antimicrobial and oxidation resistance properties) and physiochemical (barrier property, pH, etc.) traits that help to overcome some of the drawbacks of gelatin composite film and broaden its potential for use in food conservation and future industrialization. Gelatin film can currently be made through coating, casting, extruding, and electrospinning, although it is still difficult to create films using extrusion molding.

When compared to mammalian gelatin, aquatic gelatin (also known as fish gelatin) is a significant hydrocolloid of food grade that is obtained from aquatic sources. Gelatin, which is naturally composed of protein, carbohydrates, and phenolics, is a promising biological material that has gained a lot of courtesy in current years.

In recent years, the utilization of natural diet supplements with antibacterial and/or antioxidant characteristics deprived of detrimental impacts on social health has expanded in an effort to decrease the usage of synthetic chemical food additives. These organic additions can stop or lessen food deterioration brought on by

oxidation or microbiological impacts, preserving and prolonging food shelf life (Atarés and Chiralt 2016).

To ensure the film's stability in humid circumstances for food packaging applications, the gelatin structure must be preserved. Cross-linking has been done using glutaraldehyde, but it has a significant drawback in that it is a systemic and cell hazardous chemical (Biscarat et al. 2015). He also created substitute matrices based on gelatin type A with some other chemicals which are less harmful to manufacture films by dry casting in order to avoid using glutaraldehyde (Biscarat et al. 2015).

The development of cutting-edge food packaging technologies with smart materials in gelatin matrices that extend the safety and superior quality of food products has been the focus of the current research. For instance, Musso et al. created biofilms by casting glycerol, bovine gelatin, and three pH-sensing acid-base indicators such as methyl orange, neutral red, and bromocresol green (Musso et al. 2016).

Vegetables

Vegetables are preserved with gelatin. Vegetables are a great source of fiber, vitamins, and minerals. These elements are crucial to a healthy eating routine. However, owing to their high humidity content (75–95%), vegetables have a short shelf life and spoilage and degradation happen quickly (Arfat et al. 2014). Fruits and vegetables control inhalation and exhalation rate to modify O₂ and CO₂ concentrations and provide comparatively steady metabolic processes. Freshly selected veggies must be packaged and coated since they are susceptible to bacterial, yeast, and mold-related deterioration (Plackett 2011).

Due to the incessant use of ethylene maturing agents, overripening, decrease in water content, and rot cause the appearance and quality of fresh fruits to degrade during the processes of picking, storing, and transportation. The ability of gelatin-based edible films and coatings to persist the shelf life of various fruits has been tested.

Aquatic Products

Aquatic goods are unpreserved because they have significant levels of polyunsaturated fatty acids, proteins, free amino acids, and endogenic enzymes that make them susceptible to oxidation and microbial degradation (Arfat et al. 2014).

As a result, the food becomes rotten and discolored. Aquatic items can be given an edible coating to increase product quality and shelf life (Pirayesh et al. 2013). Because of its excellent film-forming and glutinous qualities, as well as its low aroma and excellent barrier capabilities, gelatin is frequently employed in food packaging (Valdés et al. 2016).

Meat

The human diet's high-value food source, meat, contains a number of the important fatty acids that the body needs. However, meat products are susceptible to spoiling and depreciation during production, processing, and storage, and the presence of microbes and endogenous enzymes will alter the freshness of the meat (Moon et al. 2011). To preclude the growth of microbes and preserve the textural features of

meats, edible films and coatings which are gelatin based are now frequently used in the preservation of meat products. This is done by changing the gelatin-based packaging.

When gelatin film was combined with nanochitosan and essential oil, the outcomes demonstrated that the well-treated chicken meat displayed preferable remarkable features such as color, taste, and odor during the entire storage time (Rhim and Kim 2014). Comparing the samples enveloped in pure poly(lactic acid) with the control, the antibacterial activity was also markedly improved. Various gelatin matrices utilized in the coatings and edible films for meat and fish items are tabulated in Table 2.

Baking

Foods (bread, cakes, and biscuits) that are baked are rich in nutrients (protein, fat, fiber, and sugar). They quickly oxidize and grow mold, which damages the product. Mold-induced food spoiling can cause significant financial losses and release dangerous consequential metabolites (mycotoxins) which have an effect on human health. In order to retain the desired qualities of bread quality, papain is added to the gelatin film. This procedure boosts the volume of bread and improves the quality of frozen dough bread. After 60 days of frozen storage, the amount of thiol groups (-SH) and the degree of depolymerization of the glutenin macropolymer both significantly decreased, but there were no appreciable alterations in the secondary structure delaying the harmful deterioration of the frozen dough. The frozen dough might be efficiently protected by gelatin and papain (Hosseini et al. 2015; Panzavolta et al. 2014).

Biomedical Applications of Gelatin

Gelatin in Pharmaceutical Industry

Gelatin serves as a significant and effective ingredient for pharmaceutical and medical applications. It is widely utilized in these applications as a consequence of the fact that it exhibits excellent properties like nontoxicity and hypoallergenicity, exceptional biocompatibility, good solubility in biological fluids at body temperature, high purity, ability to form a robust film having a homogeneous structure, limited serological activity, and amenable physical characters.

A significant quota of gelatin consumed by the pharmaceutical industry is utilized primarily for hard and soft gelatin capsules (softgels), as well as tableting, tablet coating, granulation, encapsulation, and microencapsulation, since it limits oxidation and enhances the palatability of the composition (Eaton 1989; Nishimoto et al. 2005; Stringer 1989; Withered 1986).

Correll et al. reported the formation of a nonantigenic, sterile, absorbable, and water-insoluble gelatin film (Gelfilm™) using a specially prepared gelatin/formaldehyde solution (Correll 1950). In the encapsulation of the fat-soluble vitamins, gelatins with bloom values ranging from 0–140 are advised. Fish gelatins are commonly used for microencapsulation since they have exceptional film forming

Table 2 Various gelatin matrices utilized in the coatings and edible films

Food applicability	Product	Matrix	Processing method and final product	Reference
Meat products	Angus steaks	Chitosan and bovine type B gelatin together	Entering matrix solution (coating)	(Cardoso et al. 2016)
	Boar sausages	Blends of gelatin, pectin, and sodium alginate	Extrusion (film)	(Liu et al. 2007)
	Lamb loin	Animal gelatin	Penetrating the gelatin matrix (coating)	(Herring et al. 2010)
	Dry sausages from Kabanosy	Glycerol, kappa carrageenan, and pork gelatin	Entering matrix solution (coating)	(Tyburcy and Kozyra 2010)
	Chicken breasts	Gelatin made from skate skin and essential thyme oil	Casting (film)	(Lee et al. 2016)
	Fresh beef	<i>Thymus vulgaris</i> and <i>Rosmarinus officinalis</i> essential oils are combined with gelatin and Tween 80	Entering matrix solution (coating)	(de Oliveira et al. 2013)
	Turkey bologna	Nisaplin, glycerol, gelatin, and the antibacterial additives Guardian CS1-50	Casting (film)	(Min et al. 2010)
	Bacon	Gelatin	Casting (film)	(Nowzari et al. 2013)
Fishery products	Rainbow trout	Chitosan, glycerol, and fish skin from cold water	Casting and dipping into matrix solution (film and coating)	(Gómez-Estaca et al. 2010)
	Cod fillets	Clove essential oil combined with bovine hide gelatin, chitosan, sorbitol, and glycerol	Casting (film)	(Nowzari et al. 2013)
	Minced fillets of trout	Red grape seed extract, cold water fish skin gelatin, chitosan, glycerol, and <i>Ziziphora linopodioides</i> essential oil	Casting (film)	(Kakaei and Shahbazi 2016)
	Filletts of rainbow trout	Laurel leaf essential oil, glycerol, sorbitol, food-grade gelatin, and sorbitol	Casting (film)	(Alparslan et al. 2014)
	Tuna meat	Several plasticizers, gelatin, and protein from red pepper seed meal	Casting (film)	(Lee et al. 2016)
	Fish sausages	Gelatin from warm water fish, chitosan, concentrated shrimp, Tween 80, and glycerol	Casting (film)	(Alemán et al. 2016)

(continued)

Table 2 (continued)

Food applicability	Product	Matrix	Processing method and final product	Reference
	Atlantic salmon	Gelatin, lignin, sorbitol, and glycerol	Casting (film)	(Ojagh et al. 2011)
	Salmon	Barley bran protein, grapefruit seed extract, sorbitol, and porcine skin gelatin	Casting (film)	(Song et al. 2012)
	Cold smoked salmon	Chicken feather protein, sorbitol, clove oil, and pork gelatin	Casting (film)	(Song et al. 2014)
	Shrimps	Tween 20, orange leaf extract, sorbitol, glycerol, and gelatin	Entering matrix solution (coating)	(Alparslan et al. 2016)

properties (Morimura et al. 2002). The earlier comments regarding gelatin capsules does not refer to any of the constituents other than the medications encapsulated. Current constitutions, on the other hand, contain a wide range of other ingredients, each with their own advantages (Gold et al. 1997).

Gelatin capsules are frequently used for the encapsulation of a diverse range of foods, nutraceuticals, and drugs. The encapsulated materials are secured from humidity, temperature, or other drastic environments, improving their durability and sustainability, and this has led to a boom in applications for this procedure (Gibbs et al. 1999). Gelatin is used as an excipient in medicinal preparations like vaccines and tablets (Sam 2000). Gelatin films derived from the skins of warm water fish species such as *Lates niloticus* (Nile perch) manifested fracture strain akin to gelatin obtained from the bone of cattle (Muyonga et al. 2004). However, fish gelatin film demonstrates a lesser permeation of water vapor than bovine and pigskin gelatin (Gómez-Guillén et al. 2007).

Gelatin as Tissue Engineering Scaffolds

Gelatin is a commonly used biopolymer for the generation of scaffolds, with the ability to replace native extracellular matrices (ECM) (Tayebjee et al. 2003; Jiang et al. 2014). For a material to be used as a scaffold in tissue engineering, three basic prerequisites must be fulfilled: (1) the substance that holds up the matrix must be compatible biologically and capable of being molded into the intended shape; (2) the relation between the substance and the host cell must comply with tissue-specific constitutional and metabolic requirements; and (3) the matrix's performance must be appraised within the living organism as well as in the artificial environment by quantifiable molecular and histopathological diagnostics (Langer and Vacanti 1993). Gelatin fulfills these requirements as it is biodegradable, has good cell proliferation capability, and has low immune response (Hayashi et al. 2012; Horch et al. 2000; Pati et al. 2012).

Since gelatin is manufactured from collagen by means of hydrolysis, the cleavage of intermolecular bonds leads to the separation of the chains in the α -helical structure. However, the discrete chains retain their primary structure, which offers the RGD amino acid motif (arginine [Arg]–glycine [Gly]–aspartic acid [Asp]), which is the identification array for cell adhesion facilitated by integrin (Yamada et al. 2014). Gelatin, when used as a scaffold material, enhances permeation, binding, spreading, and multiplication of cells on the scaffolds. Microspheres of gelatin, cross-linked using genipin, exhibited enhanced proliferation of exceedingly viable hepatocellular carcinoma cells (Lau et al. 2011).

A better and refined outline is a cell microcarrier with opened, vacant, and casing-like structure developed using gelatin, by cross-linking strategy and further solubilizing the substances which were not cross-linked (Su et al. 2011). The system had a higher loading efficiency and good compatibility, which supports cell binding, faster multiplication, and well-regulated differentiation.

Natural biopolymers have a favorable influence on signal recognition, allowing the cells to penetrate deeper within the scaffold. Furthermore, the creation of new cavities or extension of the already prevailing ones is expedited by the increased elasticity and deformability. Hydroxyapatite (HA), a mineral present in the nature, which makes up a part of the bone, has been coated with gelatin to construct a composite with improved mechanical properties. Because gelatin increases toughness by bridging material cracks in HA, the refinement in properties was found to be proportional to the amount of gelatin in the composite (Dressler et al. 2011).

Composites of gelatin integrated with other natural polymers such as collagen, hyaluronan, chitin, and silk have good biocompatibility and can be employed in biomimetic strategies (Kundu et al. 2014; Wan and Tai 2013). The composites of hyaluronan and gelatin have been constructed to harvest the beneficial properties of both these materials. While hyaluronan is a polysaccharide with excellent water retention and numerous binding sites for molecules, its hydrophilicity deters protein adsorption and in consequence prevents binding of cell. The incorporation of gelatin with hyaluronan was found to increase the cell proliferation (Park et al. 2014). Silk was used to fortify gelatin scaffolds, which resulted in higher tensile and deforming strength (Zhang et al. 2015).

Gelatin has also been combined with agarose or alginate to develop a phase transfer cell culture (PTCC) system, with gelatin serving as the porous material with prolonged solubility (Gong et al. 2010; Su et al. 2012). For the transplantation of therapeutic cells, a 3D scaffold-free living gristle graft was also created (Lau et al. 2012; Su et al. 2012).

Man-made macromolecules have the advantage of tuneable and modifiable material chemistry. But most of them lack cellular identification arrays and attachment sites (Toh and Loh 2014). Exploiting gelatin's cell recognition ability and binding sites in the form of composites with synthetic polymers can be beneficial for engineering scaffolds. The composite designed by combining gelatin and polyamide exhibited cell recognition as well as biomechanical properties. When transplanted into rats, it also promoted wound healing (Ulrich et al. 2014).

Ozkizilcik and Tuzlakoglu developed gelatin-poly(lactic-co-glycolic acid (PLGA) composite particles, and the chondrocytes cultured on these exhibited enhanced proliferation, viability, and deposited glycosaminoglycan more than PLGA alone. This system can be injected and has the potential be used to deliver chondrocytes to articular cartilage defects, which are very common in clinical practice (Ozkizilcik and Tuzlakoglu 2014). Electrospinning technique can also be employed to combine PLGA and gelatin. The composite fibers outperformed PLGA alone in terms of cell adhesion and proliferation. Incorporation of gelatin into PLGA facilitates tunable release kinetics and improved biocompatibility (Meng et al. 2010).

For the treatment of the injury of cartilage and underlying bone in rabbits, gelatin was stocked with either insulin-like growth factor 1 (IGF-1), TGF- β 3, or both and were incorporated in the gristle section of a bilayered oligo(poly(ethylene glycol) fumarate) (OPF) design. This composite was able to discharge growth factors for 25 days in vitro (Kim et al. 2013). Gelatin-OPF composite could potentially be used for the regeneration of tissues such as the interface between bones and cartilage.

Gelatin in Drug Delivery

Biodegradable polymers like polysaccharides and proteins can be used as effective delivery systems for the structured and targeted drug release, with the intention of improving the efficacy and minimizing the complications of the medicines (Kumari et al. 2010). Inclusion of gelatin within other matrixes can offer systems for the delivery of therapeutics. These have the combined properties of both the materials making them excellent agents for drug delivery.

Nanoparticles of gelatin are also utilized to deliver protein and peptide-based therapeutics. Gelatin nanoparticles encapsulated in PLGA and loaded with bovine serum albumin (BSA) indicated sustained release features, along with the ability to arrest the denaturation of protein (Tang et al. 2012). Won et al. investigated recombinant human gelatin (rHG) nanoparticles for delivery properties using fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA). The sustained release of rHG nanoparticles was accompanied by a lower initial burst. Other protein drugs, such as insulin, bone morphogenetic protein-2 (BMP- 2), alkaline phosphatase (ALP), tissue-type plasminogen activator (t-PA), and angiogenic bFGF, were also incorporated into gelatin nanoparticles, with the result that the efficacy of these drugs was maintained within the living systems (Won et al. 2012).

Gelatin nanoparticles are also widely exploited for the discharge of both water-soluble and water-insoluble antineoplastic agents, with the goal of prolonging discharge, improving the targeting efficacy, and reducing toxicity. The advantages of gelatin nanoparticles as antineoplastic drug delivery agents are very low cytotoxicity, reproducible synthesis, and low cost. Gelatin nanoparticles also possess passive targeting ability, as result of which they remain at site of the tumor for longer periods so as to facilitate the complete release of the drugs. Gelatin nanoparticles loaded with the anticancer drug Paclitaxel exhibited approximately 2.6 times higher concentrations in tumor-affected cells when correlated with that loaded in the Cremophor-ethanol (CrEL) vehicle following intravesical administration in the dogs (Lu et al. 2004). The exceptional efficacy of antineoplastic agent-loaded gelatin

nanoparticles as correlated to free agents was revealed both *in vitro* and *in vivo*. An increase in the tumor volume to about 20 times relative to the initial volume was observed in mice medicated with free 17-allylamino-17-demethoxygeldanamycin (17-AAG), in comparison to about 15 times in 17-AAG loaded in recombinant human gelatin modified with alpha-tocopheryl succinate (rHG-TOS) (Won et al. 2011).

Doxorubicin loaded into glutaraldehyde cross-linked gelatin nanoparticles injected into peritoneal cavity of rats increased the efficacy of the anticancer agent. However, repeated administrations were found to cause notable cardiotoxicity (Kommareddy et al. 2007).

In addition to anticancer and protein drug delivery, gelatin nanoparticles are employed for the discharge of a variety of chemotherapeutic agents like antiviral, antimalarial, antifungal, antitubercular, anti-inflammatory, analgesic, antispasticity, and antispasmodic agents and antidiabetic agents. In all these cases, gelatin nanoparticles could prolong the drug release, reduce the side effects of the drugs, and improve the efficacy of the drugs. Gelatin nanoparticles could prolong the release of the antimalarial agent cryptolepine to as long as 192 h (Kuntworbe and Al-Kassas 2012). The incorporation of the antitubercular antibiotic rifampicin into gelatin nanoparticles led to the improved assimilation of the drug by the cells in the lungs, thereby increasing its availability and efficacy in mouse infected with tuberculosis (Saraogi et al. 2010). The loading of the antifungal antibiotic amphotericin B and antimalarial agent cryptolepine into gelatin nanoparticles could reduce the nephrotoxic and haemolytic effects of the medicines, respectively (Kuntworbe et al. 2013).

Cosmetic Applications

Gelatin is a key component of cosmetic products because of how well suited its qualities are for encasing bath oils and for usage in moisturizing lotions and skin creams. The cosmetic industry has recently paid a lot of attention to gelatin films made from fish. To boost the antioxidant activity, essential oils derived from citrus fruits such as bergamot, kaffir lime, etc. were added to fish gelatin films (Tongnuanchan et al. 2015). Gelatin hydrolysates are crucial ingredients in skin care products due to their capacity to give skin firmness, suppleness, and hydration. They enhance hair shine and make combing easier in hair care products. Additionally, gelatin derived from by-products or derivatives of pigs and cows is used in cosmetic items. Many cosmetic products, such as face creams, body lotions, shampoos, hair sprays, sunscreens, bath salts, and bubble bath, use gelatin as a gelling agent. The primary function of the skin is to protect the body from the elements, especially sunlight. Free radicals are produced during the oxidative process of skin aging. The skin does, however, have a built-in antioxidant defense system. Melanin, the skin's natural pigment, absorbs ultraviolet (UV) light (Aguirre-Cruz et al. 2020). Fish gelatin hydrolysates were utilized for the protection from UV radiation and to enhance water binding ability and minimize dehydration. They restored the skin's structural integrity by keeping the skin's lipids in equilibrium because of their

antioxidant qualities (Al-Nimry et al. 2021). Antioxidant enzymes in the body, which shields the skin from oxidative stress, are reduced as a result of UV radiation exposure. Studies on mice were conducted to ascertain the antiaging and antioxidant effects of polypeptides produced from Pacific cod (*Gadus macrocephalus*). These studies revealed that gelatin hydrolysates considerably boosted the activity of antioxidant enzymes. On experimental mice, oxidation of lipids was also decreased, and levels of inflammatory cytokines were downregulated. This may be because NF- κ B was inhibited. In a different study, the protective properties of salmon fish gelatin and its hydrolysates were assessed on mouse skin. Lipid peroxidation was reduced and down again, it was discovered that the levels of antioxidants enzymes were raised by gelatin and its hydrolysate, reducing the negative effects of UV light (Chen and Hou 2016). Gelatin and its hydrolysates also increase immunity by increasing the thymus index and hydroxyproline in the skin, which serves as a marker for the collagen content, in order to defend against UV radiation. The tilapia (*Oreochromis niloticus*) gelatin hydrolysates were discovered to have a scavenging action against reactive oxygen species generated by UV radiation which harm skin and induce early aging (Sun et al. 2013). Therefore, fish gelatin/hydrolysates could be viewed as a unique source of ingredient with potential for use in skin antiaging products in the future.

Environmental Applications

Huge quantities of industrial effluents containing dyes are mainly produced by textile, leather, food, cosmetics, and polymer industries. Effluents containing dyes can affect the aquatic life by preventing the penetration of sunlight and oxygen. Humans also get affected due to the fact that dyes are not biodegradable and deposit in the body, causing diseases like cancer and skin allergies. Gelatin-based composite materials are widely used in the adsorption of dyes, hazardous metals, phosphate and nitrate, and greasy pollutants. To improve the efficiency of adsorption, different materials such as chitosan, carbon nanotubes, calcium alginate nanofiber membranes, acrylamide and 4-styrenesulfonic acid sodium salt, etc. are incorporated into gelatin-based composite materials (Rigueto et al. 2021).

Removal of Dyes

The process of adsorption of dyes is dependent on pH parameter used in the solution. pH value has an impact on the surface charge of the adsorbent and the structure of the dye molecules (El-Aassar et al. 2018). As a result, it is governed by the characteristics of the dye under investigation and adsorbent's zero-point charge (pH(PZC)). When pH is greater than pH(PZC), cationic dyes are more likely to adsorb, whereas anionic dyes are favored at a lower pH than pH(PZC) (He et al. 2015). As reported by Wang et.al, amphoteric carboxymethyl chitosan/gelatin microspheres were less effective in adsorbing methyl orange (anionic dye) with decrease in pH and an opposite trend was observed with methylene blue (cationic dye) (Wang et al. 2020). Similar behavior was observed when methylene blue dye was adsorbed by fibrous

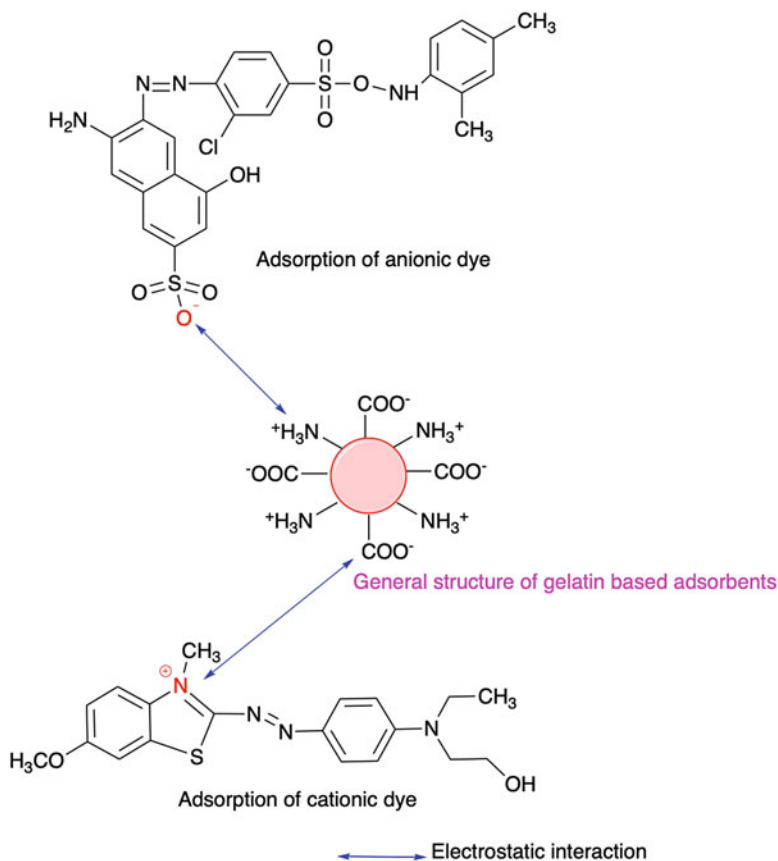


Fig. 5 Process of dye adsorption by gelatin-based adsorbents

sepiolite clay-incorporated gelatin biopolymer. The adsorption capacity was enhanced from 195 to 467.4 mg. g⁻¹ when pH of the dye solution was raised from 4–12 (Sabzi et al. 2019). The electrostatic interactions between the cationic dye and the anionic composite can account for this. Figure 5 depicts the schematic representation of the process underlying in the dye adsorption by the gelatin-based adsorbents.

The electrostatic interaction between gelatin and dyes governs the adsorption of dyes. The pore size, surface volume, and other peculiar features of the adsorbent also determine the adsorption. Additional processes that contribute to the adsorption include sorption, cation-anion exchange, dipole-dipole interactions, and hydrogen bonding.

Removal of Toxic Metals

The metals most frequently identified in industrial effluents, according to Barakat, include cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), arsenic (As),

lead (Pb), and zinc (Zn) (Barakat 2011). Toxic metals can harm the environment and harm both marine and terrestrial biotas because of pollution of rivers, lakes, soils, and use of contaminated water by animals as well as human. The human body can suffer considerable harm from toxic metals, ranging from minor symptoms like headache and diarrhea to cancer (Barakat 2011). According to Perumal et al., gelatin and chitosan hydrogel particles that were embedded in graphene oxide led to the elimination of more than 75% of metals like Pb (II), Cd (II), Hg (II), and Cr (III) (Perumal et al. 2020).

A hybrid aerogel of silica and mesoporous gelatin created by Herman et al. could eliminate 91% of aqueous Hg (II), in the presence of additional metal ions like Cu (II), Cd (II), Co (II), Pb (II), Ni (II), Ag (I), and Zn (II). Equilibrium was achieved in 15 min and was possible to recycle the adsorption capacity five times without suffering appreciable losses (Herman et al. 2019).

In addition to groups like imidazole and thiol, amino groups also significantly contributed to the removal of metals like Cd (II) and Pb (II), employing composites made from a gelatin solution with polyethyleneimine (Li et al. 2016). Similar to the previous study, Wang et al. (2018) investigated the elimination of Cd (II) and Pb (II), but they did so using a mixture of gelatin and manganese dioxide. The success of their method was due to the presence of amino, imidazole, and thiol groups in the adsorbent (Wang et al. 2018).

Removal of Phosphates and Nitrates

The scientific community has investigated gelatin-based composites for the removal of phosphates and nitrates from aquatic matrices. Kumar and Viswanathan developed gelatin composites which has high adsorption capacity for phosphates and nitrates and reusability upto four cycles (Kumar and Viswanathan 2019). Among five adsorbents developed, Fe₃O₄ gelatin composite has shown greater phosphate adsorption efficiency above 75%. Organophosphate pesticides from waste water could also be removed using gelatin-based adsorbents.

Removal of Oily Contaminants

Oily effluents are produced in a wide concentration range by the manufacturing sectors such as food industry, textile mills, paper mills, and metal industry. These effluents can take on different forms, including fats, lubricants, chemical additives, and hydrocarbons. The petroleum industries are also responsible for wastewater with petroleum and hydrocarbon derivatives. Many studies have been developed in this field for the recovery of oily contaminants from wastewater. Various adsorbents like activated charcoal, sawdust, vermiculite, and other biopolymers have been reported to be effective in removing oily contaminants. But biopolymers, like gelatin, are desirable since they allow for chemical changes through the addition of other components (Elanchezhian et al. 2018).

Gelatin-based ferrogel composite with 15% gum Arabic has been found to have high oil retention stability and was good up to the fourth cycle. Nevertheless, around 60% of the oil was lost subsequently.

Photographic Applications

Gelatin is an extensively used material in the photographic industry. It can be utilized to make paper and photographic film. Additionally, it can be used to create photo-sensitive materials. It is a crucial component of photography supplies. It makes up a sizable portion of photoemulsion films; for example, for color print papers and color-negative films, it accounts for around 60% and 40% of the weight, respectively. Extremely sensitive photographic emulsions have developed as a result of the demands of modern photography. The photographic emulsion is without a doubt the most well-known sensitive substance (Mees 1954; Walls and Attridge 1977). Silver halide, the emulsion's light-sensitive component, should be utilized with gelatin as a binder. Although various binders, such as polyvinyl alcohol, have been employed, gelatin is the best. Gelatin for photographic purposes is often derived from pig skin and cattle bones (Ward and Courts 1977).

Gelatin's Function in the Emulsion

When exposed to light, silver chloride and silver bromide in the combination of a photographic sheet discolor and break down into their component elements. This blackening is a relatively gradual process unless chemically augmented by the use of a "developer," which releases silver from the uncovered grains without harming covered grains. The majority of the energy required to create an image comes from this process. The manufacture of emulsion is important to take into consideration, because the qualities of an emulsion, which usually often involve gelatin as the primary colloid, are critical in determining the condition of the picture acquired.

Emulsion Production

The first step in the synthesis is precipitation of silver chloride or silver bromide precipitate or crystals from alkaline bromides and silver nitrate. The reaction is conducted in a colloidal media in order to maintain the precipitate of silver salts.

The combination then goes through a process of physical evolution or aging, during which the precipitates of silver salts enlarge. After that, the residual soluble components are washed out of the solution. This process required gelling the colloidal suspension over a long period of time followed by dialysis of the soluble components by cleansing the gel in icy water. Nowadays, the majority of producers rely on salts or organic reagents, such as modified gelatins for the flocculation of colloid, which flocculate in an acidic environment. The second stage is a chemical ripening. The creation of reactive centers on the precipitate of the silver salts is now stimulated by a few crucial additions. The ripening process is stopped, the emulsion's covering is stimulated, the emulsion is hardened, and then the emulsion is rendered insoluble to the developer by the employment of various chemicals. The emulsion is then chilled after being overlaid into its support. It is protected with a coating of gelatin that will not interfere with photographs (Lestra 1985).

What Gelatin Does

The colloid of choice for making emulsions is typically gelatin. The silver salts are kept in suspension during precipitation and physical ripening by this substance, which is also significantly immersed at the surface of the precipitate of silver halide or crystals, slowing the growth of those crystals. Gelatin's propensity to gel makes it possible to remove undesired soluble components by dialysis and its elevated stickiness makes it easier to coat the support with the emulsion (Park et al. 2001). After coating, the gel that molded keeps the precipitate or crystals of silver salt on the support during dehydrating and development. The gelatin stops the counterreaction of photolysis by fixing the released halogen when it is exposed to light. Gelatin also inhibits declining of the latent image obtained from the photolysis. Reagents can infiltrate through the emulsion layer during development because the additional reagents cause the gelatin to swell. Quick development at elevated temperatures is made possible by the gelatin's ability to tan with numerous substances.

Since bone or skin collagen is used to extract gelatin, most preparations inherently include contaminants. Heavy metals, "fogging agents," dust, and grease are some that must be eliminated since they are harmful to the photographic process. As "restrainers," albumens, polypeptides comprising cystine, and nucleic acids prevent the emergence of reactivity sites or the development of silver halide particles. However, some adulterations have advantageous characteristics that serve as "sensitizers" and aid in the development of silver or silver sulfide centers on the halide particles.

Gelatin in photographic application can be divided into four groups. First one is inert gelatin, which lacks sensitizers and restrainers. Second one is active gelatin, which contains a lot of sensitizers but little restrainer. Third one is retarded gelatin, which contains a lot of restrainers but little sensitizer and the last one is retarded active gelatin, which contains a lot of both.

Finding the best gelatin for a specific application is the responsibility of the manufacturer of photographic emulsion (Gelatin Handbook 2012). In order to preserve quality from batch to batch, the gelatin maker must produce the gelatin according to strict specifications.

Production of Gelatin

The conditions of gelatin extraction determine the attributes of the ultimate product. The liming activity in precise requirements are to be carefully monitored. The triple helix of collagen is broken down at this step due to the hydrolysis of amide groups into carboxylic groups, the removal of numerous impurities, including sulfur, the conversion of the sulfur in cystine to thiosulfate, and the breakdown of RNA into nucleotides. The original material is limed and then treated in aquatic environment to remove as much lime as viable before being "delimed" in a bath of diluted acid. Then, only the first extraction of gelatin is pure enough to meet the stringent standards of photography. By merely reducing the pH, gelatins can be chemically altered to cause flocculation. This type of gelatin is being used more frequently to flocculate emulsions in place of sodium or ammonium sulfate or dialysis-based washing.

Auxiliary Gelatins

Unlike other materials, photographs do not have just one light-sensitive layer. A further auxiliary layer is placed to the support either after the emulsion layer or concurrently. The hydrophilic emulsion has little affinity for the support's surface and is often synthetic. It is vital to have an intermediary layer, or substratum, that contains the components required to create a link between the emulsion and support. Each layer in multiple emulsions is sensitive to a different radiation band which is present in color films or sheets. To stop diffusion from one color layer to another, intermediate layers must be used between the layers.

The emulsion layer's surface needs to be mechanically shielded from outside touch. The supercoat serves this purpose. It must have no photographic impact on the emulsion, like all auxiliary layers. Typically, they are inert gelatines.

To balance off the tension that all the layers place on one side of the support, a backup layer is superimposed on the opposite side. This is frequently colored to elude the halo that the light ray produces when it replicates on the film. Up to a dozen chemical layers may be present in several color movies. At speeds of 100–150 m/min, the sophisticated coating equipment currently in use enables the synchronized coating of the majority of the layers. Since the thickness of individual layer is just about 10 μm , it is simple to see the significance of the gelatin's substantial requirements.

To provide the customer with photographic gelatin that has exactly the attributes they require, the producer must be able to regulate the manufacturing process very accurately and uphold stringent quality regulator. When defining the specifications for an emulsion, it is also helpful for the client to understand the manufacturer's challenges.

Gelatin is used as the dispersion medium for the light-sensitive silver salts in the gelatin process, commonly known as the gelatin dry plate technique. The technique, which was developed around 1880, replaced the wet collodion method, in which a glass plate was coated with nitrocellulose (collodion) solution just before exposure to create a wet negative.

Wherever a photograph was to be taken, a darkroom was required for this chemical process. A method for drying a sensitized gelatin emulsion on a plate and keeping it there for months before use, sheltered from light, revolutionized photography. The standard binding substance for the silver halide crystals used in common photographic film is still gelatin.

In photography, gelatin is frequently employed because, after coating, the gel that is produced holds the silver salt precipitate or crystals on the support while dehydrating and development. Additionally, the gelatin fixes the released halogen when it is exposed to light, preventing the inverse reaction of photolysis. Dichromated gelatin (DCG) is an additional photosensitive material in the physics-optics areas. It has proven useful ever since it was used in the printing business more than a century ago. Making holograms is arguably the most recent successful application. In the table below, gelatin's roles in photographic materials are enumerated (Toda et al. 2002).

Table 3 illustrates the role of gelatin in various photographic materials.

Table 3 The role of gelatin in photographic ingredients

Process		Step	Functions expected from gelatin	Characteristics or properties of gelatin
Production process	AgX emulsions	Nucleation (Tabular)	Encourage the creation of parallel twin planes and discourage random coalescence	Adsorption behavior on AgX crystal surfaces, molecular weight, methionine concentration, and gelation temperature
		Growth	Growth regulation avoids aggregation	Adsorption behavior, inertness, reducing activity, and molecular weight on AgX crystal surfaces
		Demineralization	Bring about permanent flocculation	Adsorption behavior and interactions with flocculants on AgX crystal surfaces
		Chemical maturing	Not obstruct the ripening process Avoid accumulation	Adsorption that is competitive with ripener species, inert, and reducing in nature
		Repository	Refrain from sedimentation	Reversible gelation and viscosity
	O/W emulsions	Emulsification	Mechanical energy propagation and interfacial energy reduction	Viscosity and surfactant interaction
		Repository	Halt emulsion deterioration	Surfactant collaboration, viscosity, and reversible gelation
	Film formation	Coating	Maintain laminar flow while stabilizing a rapidly expanding surface Wet and applied to a specific substrate	Viscosity, interactions with ionic polymers, and interactions with surfactants
		Drying	Keep the layered structure and provide a reliable matrix	Gelation, cross-linker reactivity, and moisture content
	Finishing	Slitting	Protect the system	Gel durability and elasticity
Post-production process	Exposure		Maintain the latent image not to impede the optical process	Transparency, interaction with halide ions, and the rate of expansions and shrinkage as a function of humidity

(continued)

Table 3 (continued)

Process		Step	Functions expected from gelatin	Characteristics or properties of gelatin
	Development		Give the procedure an aqueous medium	Cross-linkability and swelling
	Preservation		Maintain and safeguard the image	Cross-linkability, oxygen (im) permeability, water absorption behavior, and gel strength

Other Applications

Gelatins used for applications other than food and biomedical applications need not have the rigorous specifications meant for human consumption. Gelatin can be used as a mid-IR recording medium. Visible light can pass through gelatin with ease. However, mid-infrared light transmittance for films with a thickness of about 90 m is only about 10% (= 10.6 m). Mid-infrared light is heavily absorbed by thicker layers. Surface relief gratings have been made using this property. Thin gelatin films of thickness ranging from about 10 m to about 50 m were coated and were placed in a two-beam interference pattern. A CO₂ laser (= 10.6 m) was employed and the sinusoidal pattern was captured. The recording region also received light at the same time from a He-Ne laser. It was discovered that relief gratings were captured over the course of exposure time.

Gelatin can be utilized as relative humidity sensor. The thickness of gelatin varies when water vapor is adsorbed by the films. The refractive index of gelatin is 1.5 when it is dry and refractive index gets diminished when water vapor is absorbed. Thus gelatin can be used to make relative humidity sensor. Gelatin is used to make relative humidity sensors by taking into account changes in thickness and refractive index (Calixto et al. 2018).

Gelatin is utilized in paper production, printing, in the manufacture of the coated end of match, abrasives, book bindings, grooved and ribbed cardboard sealings, etc. (Green and Lowell 1957; Gutcho 1976; Wood 1977). Gelatin, along with gum arabic, is used to produce oil-containing microcapsules used in the manufacture of carbonless paper. Biodegradable gelatin adhesives are recently used. They are also used in washing and cleansing agents. Gelatin films with different colors have been used for photographic lighting.

Conclusion

Gelatin is a highly potential functional protein, which finds diverse applications. Extensive research has been carried out to identify newer sources of gelatin ranging from animals to fish and insects. It has multidimensional applications such as in food industry, pharmaceuticals, tissue engineering, controlled, targeted, and sustained

drug delivery, cosmetics, photography, and effluent treatment. It is the characteristic properties of gelatin that makes it a desirable polymer in these applications. Gelatin by itself and in combination with other natural and man-made macromolecules and ceramics have several beneficial properties. The composites of gelatin are often found to possess improved toughness coupled with lightweight and biocompatibility that makes them desirable components as biomaterials. The composites also offer greater flexibility in terms of material degradation. Current research is focused on tailoring the properties of gelatin and its composites to make them suitable for specific needs.

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Natural Rubber and Gutta-Percha Rubber

28

Applications

Hema S., Malavika Sajith, Rashid Sulthan A., Sreelekshmi C., and Sreedha Sambhudevan

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Abstract

One of the most significant polymers for human society is natural rubber (NR), which is made of long isoprene polymer chains. The sole commercial source of natural rubber latex sap is *Hevea brasiliensis*, commonly called the rubber tree, and is an important raw resource that is used to make over 40,000 different goods.

H. S. · M. Sajith · R. S. A. · S. C. · S. Sambhudevan (✉)

Department of Chemistry, Amrita Vishwa Vidyapeetham, Amritapuri, Kollam, Kerala, India

e-mail: sreedha@am.amrita.edu

NR-based composites have now widely emerged as a green composite with bio-based properties as an alternative to the rising concern over several environmental issues. Gutta-percha is a natural polymer that has many chemical qualities similar to natural rubber but differs from rubber in terms of its molecular structure. Initially created as a restorative substance, “gutta-percha” has now evolved into a crucial endodontic filling substance. In the process of developing as a specialization, it has evolved into the “soul” of endodontics. The indigenous of the Malaysian archipelago employed gutta-percha in rudimentary form for producing knife handles, walking sticks, and other things long before it was brought to the Western world. Over the years, gutta-percha has undergone a number of modifications to meet changing endodontic trends and fulfill its goals by streamlining the procedures, producing an ideal material. In this chapter, we discuss different applications of the two versatile biopolymers: natural rubber and gutta-percha rubber.

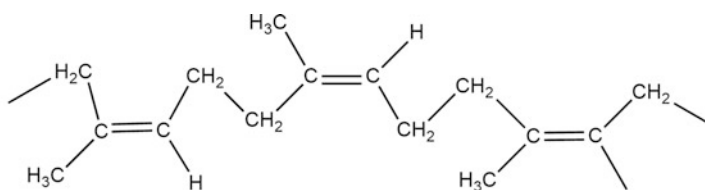
Keywords

Natural rubber · Gutta-percha · Applications · Biopolymer · Green fillers

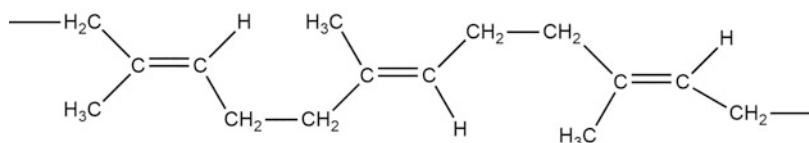
Introduction

Natural rubber (NR) is a versatile agricultural product and is found to be consumed in nearly 50,000 products worldwide. It is an elastic natural hydrocarbon polymeric product of the addition polymerization of diene monomer isoprene (C_5H_8). The uniqueness of natural rubber is that it is the only biomass among rubbers and the only hydrocarbon among biomasses that consist of carbon and hydrogen only. NR, also known as *cis*-1,4-poly(isoprene) (Fig. 2), is widely found in the milky sap or latex of *Hevea brasiliense* trees (Fig. 1), together with a tiny quantity of nonrubber compounds such as proteins, carbohydrates, lipids, and inorganic salts (Ansari et al. 2021). Natural rubber contains 0.5% water, 2.5% acetone extract, 2.5% proteins, 0.3% minerals, and 94.2% polyisoprene (Feldman 2017). Up to 90% of the world's total production of natural rubber is produced on plantations in South Asia. Due to a lethal fungal illness brought on by an infection with *Microcyclus ulei*, it can be up to 8% in Africa and just 3% in South America.

NR is popular for its flexibility, strength, and elasticity. They are resistant to water as well as certain chemicals. The loosely bounded isoprene chains reattach themselves when pulled apart, giving NR its elasticity (Kurian and Mathew 2011). Natural rubber also has several significant properties like excellent tear and tensile strength, moderate resistance to light, heat, and ozone, and high resistance to tearing, chopping, and cutting. High molecular weight and a defined molecular structure are the prime factors for the enhanced performance which cannot be mimicked by other artificial polymers. Because of its superior structural regularity and capacity to assemble molecular chains along the direction of stretching, NR exhibits strain-induced crystallization during stretching and is one of the most expensive

Fig. 1 Natural rubber tree

Natural Rubber cis-Polyisoprene



Gutta percha trans- Polyisoprene

Fig. 2 Chemical structure of natural rubber and gutta-percha rubber

elastomers and the sole biosynthesized rubber. High tensile strength, abrasion resistance, and tearing resistance are all provided via strain-induced crystallization for the rubber matrix.

Additives like activators, accelerators, and retarders have been utilized since Goodyear introduced the vulcanization to enhance the vulcanization process and mechanical qualities of rubber goods. The vulcanized rubber is far more resistant to abrasion than raw rubber and does not soften much even at very high temperatures or harden with cold water. The introduction of accelerator lends a hand to decrease the vulcanization time, temperature, and quantity of sulfur. The pace of vulcanization as well as the dispersion of created cross-links are significantly influenced by the combined usage of accelerators and zinc oxide as activator. During the vulcanization

process, rubber is heated and combined with various chemicals, such as sulfur, bisphenol, or peroxide; these chemicals are used to increase the durability and elasticity of the material so that it does not decompose too quickly. Vulcanized natural rubber (NR) has exceptional tensile strength and elongation at the break due to the strain-induced crystallization phenomena (Rattanasom and Prasertsri 2009). The homogeneous microstructure of this phenomenon, which can be called a “self-reinforcing effect,” is frequently attributed to it (cis-configuration of the macromolecular chains).

Biopolymers have garnered a lot of attention recently due to their potential to reduce the usage of fossil fuels and nonbiodegradable polymers, hence reducing plastic pollution. The majority of biopolymers are biodegradable, which means that environmental microbes may break them down into CO₂ and water. Improved biopolymer composite qualities, including those of starch-based materials, cellulose-derived polymers, bacterial polyesters, and natural rubber composites, have been the focus of recent times. The majority of current scientific research on fortifying natural rubber and enhancing its physical qualities is a serious area of interest. In order to improve the qualities of rubber and introduce new ones, fillers must be used because the properties of pure rubber are sometimes insufficient for practical applications. The presence of nanofillers in the rubber matrix results in highly effective, lightweight rubber products. Clay, carbon nanotubes, graphene, titanium dioxide, and other nanofillers are contributing significantly to the improvement of the characteristics of rubber nanocomposites. The alteration of filler surface enhances the dispersion of filler particles (hydrophilic) and rubber macromolecules in the matrix and their interfacial adhesion. This is a crucial problem since the straightforward addition of natural-organic fillers to a rubber matrix might result in inferior mechanical capabilities to the neat rubber, this is particularly true when filler particles with low-length-to-diameter ratios are utilized (Chang et al. 2021).

Gutta-percha is a polyisoprene trans-isomer; 1, 4, trans-polyisoprene is its chemical structure (Fig. 2). It has several similarities to natural rubber, which is a cis-isomer of polyisoprene in terms of molecular structure, but a variation in form causes it to act more like crystalline polymers in terms of mechanical qualities. Gutta-percha is a nonconductor that is commonly used to insulate maritime and underground wires. Golf ball covers, surgical appliances, and adhesives are all made out of it (Warneke et al. 2007).

Due to the ability to combine the advantages derived from the bio-based derivatives, NR and GP offer better flexibility and processability and have evolved as perfect polymeric blends or nanocomposites that have recently received a lot of research attention.

Natural Rubber as Biomaterials

It would be fantastic if medical gadgets could be made out of materials with biological qualities. Natural and synthetic rubber are widely employed as materials that are utilized as bodily components or functions in a safe, dependable, cost-effective, and physiologically acceptable way because of their low weight and

biocompatibility. Biocomposites are composite materials that contain one or more biologically derived phases. The use of NR as a biomaterial is rapidly expanding, considering scientific studies and material developments. Different applications of NR as a biomaterial are illustrated in Fig. 3. Both solution cast and latex films of NR have contributed biomaterials owing to their mechanical, biological, chemical, and physical features even after the process of compounding and vulcanization (Anderson 2012). Due to the presence of a double bond in the macromolecular C_5H_8 cis-configured monomer unit, NR is sensitive to oxidation and heat, and it also shows poor hemocompatibility compared to other synthetic rubbers. As it is suitable for effective processing and easy for compounding, grafting of natural rubber with improved hemocompatibility has been an emerging area of research for a long time. Another known disadvantage is that the protein latex component present in the NR system may impart allergy in human bodies. The capability to stimulate the development of new blood vessels (angiogenesis), cellular adhesion, etc. were the reasons for promoting NR as a candidate for tissue regeneration and tissue repair (Phinyocheep 2014).

Various forms of natural rubber latex (NRL)-based biomedical devices have recently been created to improve tissue healing by using the biological features of the latex, and several studies have looked at the biocompatibility of NRL composite materials. In 2021, Barros et al. synthesized NRL-alginate wound dressing materials to cure prolonged diabetic foot ulcers. Exudate release is increased when natural rubber latex dressings are applied to diabetes patients' wounds. The highly absorbent, permeable, alginate-loaded NRL dressing was designed to control humidity in wounds by absorbing excess exudate and increasing water vapor transmission (Barros et al. 2021). For years, researchers have been looking at ways to improve the bone repair process and minimize patient morbidity. GBR (guided bone regeneration) has received a lot of attention, and it was developed to help with bone healing, particularly in major bone lesions. Natural rubber-based latex and restorable membranes are the best options for the GBR approach as it does not recommend the removal and hence avoids another surgery.

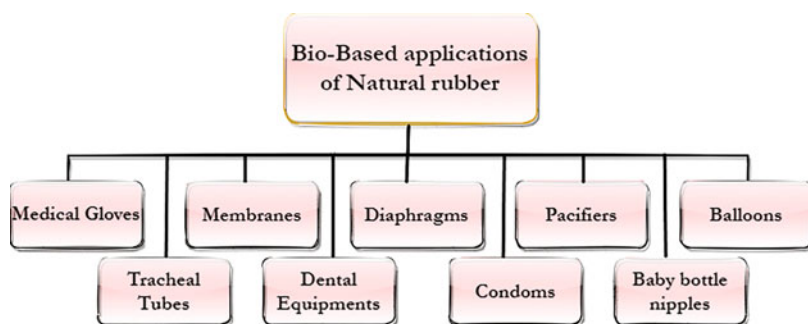


Fig. 3 Bio-based applications of natural rubber

Along with this, NRL membrane offers other advantages for human health, including avoiding bone transplants and grafts, as well as inexpensive cost, easy material accessibility, reduced pathogen transmission risk, and hypoallergenicity (Ciapetti et al. 1994; Barber et al. 2007; Guerra et al. 2021). These advancements, however, faced a variety of challenges, including rubber content and processing, characterization of mechanisms of action, purification and characterization of bio-active molecules, in vivo function, and further transformation to industry and clinical practice after regulatory approval. The application of natural rubber-based wound dressing is illustrated with a detailed mechanism in the below Fig. 4. NR composites with nanocrystalline cellulose reinforcement may soon offer improved performance, sturdiness, value, service life, and usefulness. Around 30–40% of cellulose, of which 50% is crystalline cellulose, is present in natural fibers. Although it may only be a tenth as strong as carbon nanotubes, nanocrystalline cellulose uses 50–1000 times less energy to be made. Although completely bio-based and biodegradable biocomposites can be totally recyclable and are very sensitive to moisture and temperature, they may also be more costly. Biocomposites may be 100% biodegradable if an NR matrix is employed; however, their biodegradation can only be somewhat regulated (Low et al. 2021).

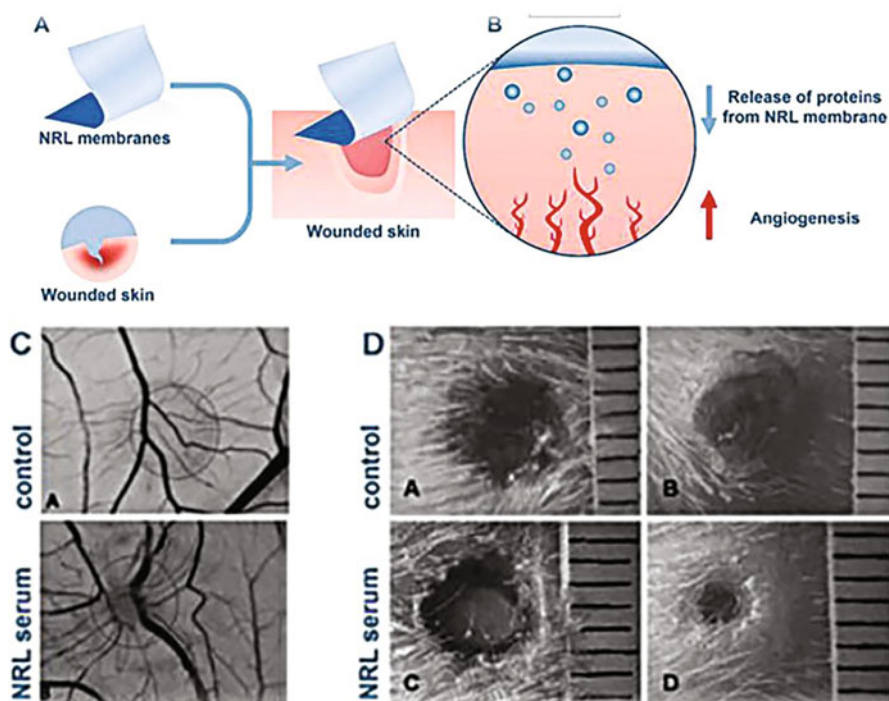


Fig. 4 Application of NR latex for wound dressing. (Adapted from Supanakorn et al. 2021 with permissions)

Regarding the cross-linking of the matrix, Abraham et al. (2012) investigated the biodegradation of NR composites enhanced with nanocellulose using the vermicomposting technique. This was accomplished by using the epigeic vermicompost worm *Eudrilus eugeniae*. The composite's reinforcing material was nanocellulose that was separated from raw jute fiber by steam explosion. A robust cellulose-NR contact is present in the cross-linked samples, according to morphological, X-ray diffraction (XRD) and tensile strength measurements. The NR composites' rate of biodegradation suggests that cellulose must first undergo hydrolysis in order for microorganisms to use it as a source of nutrients. The amount of reinforced nanocellulose in the composite greatly affects how quickly it degrades.

Natural Rubber in Transportation Sector

From ancient Mexican civilizations to modern-day tire manufacturing, the story of vulcanized rubber stretches back thousands of years. Along the way, such notable luminaries as Charles Goodyear and Charles Macintosh played a major role in its development. The majority of the output is utilized to make tires for various automobiles. Rubber production and quality are critical to the transportation and vehicle industries. Millions of automobiles are equipped with vulcanized rubber tires, which are illustrated in Fig. 5. Tire are one of the five rubber-based items that are critical to the advancement of human civilization (Thomas 2017).

Vulcanized rubber is used to manufacture all sorts of products today. Perhaps the best-known and most prevalent use of vulcanized rubber is in vehicle tires, which are commonly combined with the reinforcing agent carbon black for even greater strength. More than a billion tires are manufactured worldwide every year, making the tire industry one of the foremost consumers of vulcanized rubber. Only a small portion of natural rubber may be replaced by synthetic rubber, and the proportion of synthetic to natural rubber has decreased. Natural rubber is a substantial component of automobiles overall, but the more performance is needed, the more natural rubber

Fig. 5 Some of the rubber products used in the automobile industry



is present. About 90–100% of vehicle tires contain NR, but up to 100% of airplane and naval jet tires have NR.

In the example depicted in Fig. 6, natural rubber makes up about 30% of a tire's weight, or slightly less than 60% of the polymer component of a tire. This figure is used to be lower since more SR than NR was formerly utilized for tires. The development of the usage of radial tires and heavy-duty tires are two factors contributing to the recent increase in the percentage of NR in tires. Another significant problem in the production of tires is the unique NR odor that is released into the air during premasticating. The quality of life in neighboring residential communities as well as the job environment at manufacturing facilities may be impacted by this odor. Although the tire industry has been taking steps to deodorize the air, such as utilizing deodorant compounds or installing deodorizing machinery, there are still enough instances to demonstrate that these steps are insufficient.

Ribbed smoke sheet (RSS) and technically specified rubber are the two forms of natural rubber (NR) that the tire industry primarily uses (TSR). According to the International Standards of Quality and Packing for NR Grades, RSS is a form of "sheet rubber." In order to create 5 RSS, NR latex is first coagulated by adding acid, and then water is removed by running a ribbed surface through a mill. TSR was created in Malaysia and has been made for sale in other nations since 1965. It also goes by the name "block rubber." Depending on the grade, multiple processes are used to make TSR. The field latex serves as the only feedstock for the highest

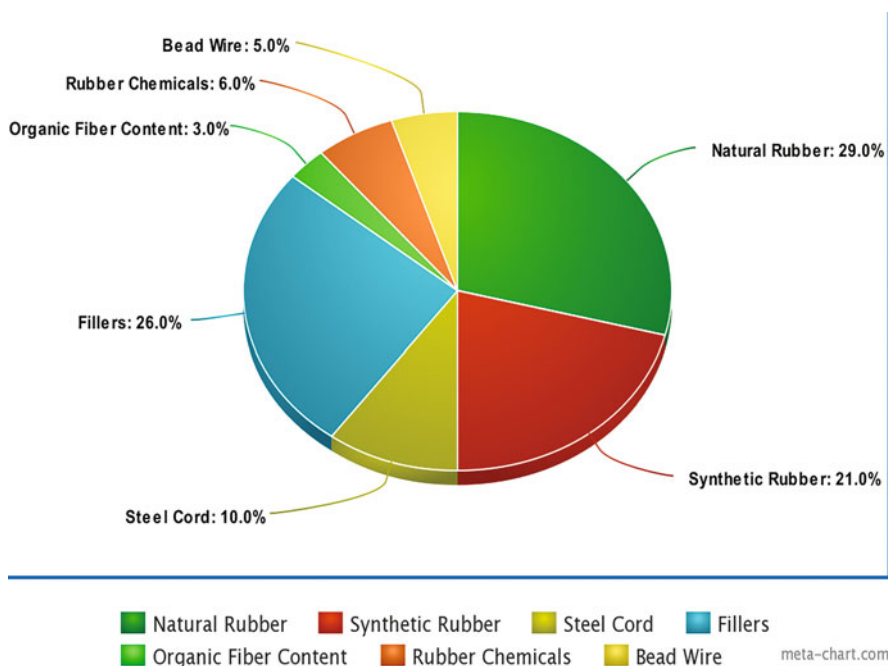


Fig. 6 Weight composition of different constituents in automobile tires

“TSR-L” grade. Other grades employ feedstock like USS, mixes, and cup lump, where the latex is acid-coagulated immediately upon tapping in the tapping cup. Because of the potential variances in feedstock quality, production methods, drying conditions, and inspection, RSS and TSR exhibit varying levels of contamination and performance (Hirata et al. 2014).

In the automobile industry, rubber and rubber products are utilized for several applications. Rubber is used in fluid transfer systems, body sealing systems, transmission systems, automotive antivibration, sealants, adhesives and coatings, O-rings, molded components, flat seals, foam and conversion goods, body parts, spare parts, and other applications in the automobile sector. The rubber sector is inextricably linked to the automotive market. Rubber’s relevance and the industry’s growth are inextricably linked. It is a well-known statistic that tires consume 75% of all the rubber produced on the planet. Both directly and indirectly, the car sector controls the rubber industry. Direct intervention refers to the original equipment tires and many other elastomeric things installed on the automobile during production, whereas indirect intervention refers to replacement items such as tires, wiper blades, and, to a lesser extent, other items (Cadwell et al. 1941).

Natural Rubber in Industrial Sector

Rubber markets have traditionally been centered in the Asia Pacific region, with industry players producing and exporting raw materials or finished goods to other countries. Rubber is utilized in a variety of industrial applications. The rubber products industry is a significant resource-based industry sector. Globally, the rubber sector has had stable and robust expansion. When we talk about the rubber industry, we are talking about a business that makes synthetic rubber and a variety of rubber items, from doormats to rubber bands to industrial rubber products. Rubber has certain distinct characteristics that make it a valuable commodity with applications in transportation, material handling, health care, and sports and leisure activities (Sitepu et al. 2019). The rubber sector provides services to a variety of downstream industries, including manufacturing, construction, and agriculture, due to the diversity of rubber goods created. Conveyor belts, power transmission belts, and other items may all benefit from the cost-effectiveness and value-addedness of isora fiber reinforced natural rubber composites.

Vulcanization paved the way for the contemporary rubber industry by allowing the substance to be used in different equipments. It is used where a high level of wear and heat resistance is required. Also, it is utilized in technical applications such as antivibration mounts, drive couplings, springs, bearings, rubber bands, and adhesives because of its strength and compressibility. However, due to its strength and heat resistance, the bulk of natural rubber (50%) is used in high-performance tires for racing cars, buses, and airplanes. Hoses, automobile components, foam mattresses, and battery boxes all utilize it (Gibbons 1939). For packing, rubberized coir sheet is used to cushion shocks and vibrations during travel. Curled coir is combined with rubber latex to create a premium packaging and upholstery material. Improved load-

bearing capacity and superior resistance to compression set are given to the fibrous constructions when natural rubber latex is used as the binder. Such characteristics guarantee that the structure quickly recovers its original shape following compression of any extent or duration, whether from shock or static stress, which is a need for any high-quality upholstery or packing material. Rubberized coir's key strength is adaptability.

Natural Rubber in Health and Hygiene Sector

Natural rubber is a versatile material that has grown in importance in the medical field in recent years. To meet rising expectations in the health industry, NR is helping to keep health care costs down by giving a cost-effective way to stay up with new advancements. Rubber has become a common base for medical supplies because of its low cost and availability. Initially, natural rubber was used, also the synthetic form of the material, silicone, became widely available, this was a bonus (Mostoni et al. 2019).

Rubber is used in a variety of medical equipments that would not function properly without it. NR-based tubing is widely utilized as a hygienic way of conveying fluids, including blood, due to its unique qualities. Rubber's elasticity makes it the best material for important medical devices like leg straps that secure fluid bags to the patient's body. Surgical gloves are constructed of a variety of natural rubber latex. These gloves have several unique characteristics that are not found in a standard glove. Ultrasensitivity, additional flexibility, maximum strength, more protection, ambidexterity, and sterilizability are some of the qualities. Medical catheters are made of rubber. A catheter is a flexible tube made of latex or silicone that may be placed into the body to allow liquids or medical devices to pass through. NR is also used to produce condoms, diaphragms, and cervical caps, all of which are effective birth control techniques (Millard 2019; de Barros et al. 2018).

Natural Rubber for Earthquake Protection

The idea of rubber mountings for protecting structures from earthquakes has been researched for a very long time. It is crucial for preventing structural damage from vibration, such as that caused by earthquakes. Theoretical studies by Lindley and innovative tests by Payne have had a significant influence on the development of vibration control technology (Pan et al. 2018). Natural rubber's great strength, minimal stiffness, linear elasticity, reversible flexibility, good adherence to metal, wear resistance, and longevity make it a very advantageous material for use in vibration isolation. We may tackle many rubber design issues by utilizing merely the value of the elastic modulus because, like other solids, rubber acts as a linearly elastic substance at small stresses and as a linear viscoelastic material at fairly large strains. Natural rubber's linear elastic properties at static and dynamic loadings, i.e., reversible deformation, are a significant benefit for the system of vibration isolation. This benefit also depends on the rubber's ability to maintain stiffness over a wide

temperature range because crystallization happens less frequently at low temperatures. Because most rubber surfaces are shielded from oxygen by bonded metals, oxygen in the environment is not a significant issue for rubber-metal laminated bearings, but ozone assault severely harms the free surface of rubber. Even at low strain levels, ozone's quick reaction to carbon-carbon double bonds results in the onset and spread of cracks extremely quickly (Kelly and van Engelen 2016; Fukahori 2014). The ideal usage of natural rubber for engineering purposes may involve a compromise between linear elasticity and nonlinear viscoelasticity.

Emerging Trend in Natural Rubber Nanocomposites

Emergence of novel nanocomposites made of natural rubber matrix with organic/inorganic fillers provides enormous opportunities for the synthesis of advanced materials with superior qualities and applications. The peak interaction of the NR matrix and nanosized fillers provides excellent qualities which pave the way for numerous applications. Various nanoparticles based on rubber matrix have been proposed to attain the desired properties like low filler loading and density, and superior chemical resistance and electrical properties. The synergy between the NR and carbon-based fillers such as CB, carbon fiber (CF), carbon nanotubes (CNTs), graphene (G), and other elastomers have been introduced into them, considering the synergistic effect of the conductive fillers and elastomers. Silicon-based nanofillers such as SiO₂, nano-layered silicates, and metal oxides have been incorporated into various polymers to improve electrical, mechanical, and thermal properties. Commercially available NR is widely used in the manufacture of adhesives, surgical gloves, tires, coatings, health care equipments and accessories, coatings, floor covering products, vehicle accessories, etc. (Hema and Sambhudevan 2021; Mahitha et al. 2020).

Inorganic nanoparticles including Si, Ag, Au, TiO₂, and other magnetic nanoparticles were added to natural rubber (NR)-based nanocomposites in order to enhance their inherent functionalities, such as their antibacterial, electrical, thermal, optical, and magnetic capabilities. For the in situ development of an inorganic shell to surround NR particles, the presence of native proteins-lipids stabilizer is advantageous. This reduces inorganic NP bulk aggregation and their incompatibility with nonpolar NR, preventing structural defects in the polymer matrix from affecting the properties of nanocomposites (Wichaita et al. 2021).

Composites' fibrous reinforcing materials might be made up of relatively short fiber segments or thin continuous fibers. Fibers having a high aspect ratio (length to diameter ratio) are used when working with short fiber segments. High-performance structural applications often require continuous fiber-reinforced composites. Continuous carbon fiber-reinforced composites can outperform typical metal fillers in terms of specific strength and stiffness (Bendahou et al. 2010). Depending on the orientation of the fibers inside the matrix, composites may be created with structural characteristics that are especially suited for a certain purpose. The NR matrix among commercial polymers must meet the following criteria, including it must be

flexible and liquid enough to readily penetrate the fabric, moisten all fiber surfaces, and separate them from one another to prevent friction between the fibers. It should also increase the mechanical strength of the finished product. In the case of composites, a chemical reaction or adsorption may be used to attach matrix molecules to the fiber surface. The surface topology of the fiber has a significant impact on the interfacial strength of composite materials (Siqueira et al. 2011). The presence of water adsorbed on the surface of natural fibers, poor wetting of hydrophilic natural fibers with hydrophobic organic polymeric matrices, thermal stresses brought on by an imbalance in the linear thermal expansion coefficients of the fiber and matrix (polymers have relatively high linear thermal expansion coefficients), and the general coating of natural fibers with waxy substances are all factors that can cause composites to delaminate.

Green Fillers for Natural Rubber Composites

Natural rubber (NR) is a versatile bio-based renewable polymer that can be employed in a variety of working environments and applications, from everyday home items to aircraft components because of its attractive properties such as lightweight, toughness, high resilience, high impact strength, tensile strength, low hysteresis, and good formation. The nonrenewable inorganic reinforcing fillers such as SiO_2 , CaCO_3 , clay, etc. are replaced by renewable organic fillers for natural rubber composites often referred to as “green natural rubber” in rubber industries for achieving the appropriate characteristics for the commercial application. The organic fillers include plants and animal remnants such as wood flour, shrimp, straw, crab shell, coconut shell fibers, etc. (Hema et al. 2021)

Plant-based and wood-derived biocomposite materials are used in many product categories including automotive, packaging, furniture, construction, and building materials. Natural fiber–reinforced composites, such as those made from kenaf, flax, jute, hemp, sisal, and henequen, are a desirable research topic because they are environmentally friendly, sustainable, inexpensive, of low density, have good mechanical properties, are simple to separate, sequester carbon dioxide, and are biodegradable. Natural fibers offer certain advantages over synthetic fibers in terms of properties and are therefore a good candidate for development into biocomposites according to their satisfactory properties (Hema et al. 2020). Also, these composites have good impact strength, stiffness, and high strength even though they came from renewable sources and are less expensive than synthetic fibers, also the health risk is low since the emission of toxic vapors during the combustion is low. Production of these fibers requires only low energy and they consume CO_2 and also recycle the O_2 in the environment. But these have some disadvantages such as low durability, high moisture absorption, and lower strength compared with the synthetic one (Masłowski et al. 2019). On the other hand, these drawbacks serve as a drive for scientists to overcome them in creating multifunctional materials.

In terms of rubber chemistry, the filler particles' capacity for dispersion inside the rubber matrix directly affects the characteristics of filler-reinforced rubber

composites. The performance of natural fiber-based rubber compounds is greatly influenced by the hydrophilic character of natural fibers. In a hydrophobic rubber matrix, it can be quite challenging to evenly spread hydrophilic natural fibers. To put it another way, it is difficult to achieve great interfacial contact between the surface of natural fibers and the rubber matrix. The first stage in decreasing the hydroxyl groups on the surface of natural fibers is surface modification, which will also lessen the hydrophilic nature of natural fibers (Ku et al. 2011). The traditional two-roll mixing mill procedure is the method used most frequently to prepare rubber composites loaded with natural fibers. In this procedure, the rubber matrix is gradually supplemented with natural fibers and other curing agents. In some instances, different surface treatments are applied to natural fibers before the mixing process in order to improve the interactions between the rubber and the fiber. From a practical standpoint, the two-rolls mixing mill operation has certain common benefits and drawbacks. Rubber sheets are created after mixing in a hydraulic press with regulated temperature and pressure. The mechanical and thermal characteristics of rubber composites are determined in the next stage.

As nanotechnology advances day by day, nano-sized renewable fillers-reinforced NR composites are becoming an exciting topic in recent trends and opportunities. NR composites having nano-sized fillers with primary particles size having diameters below 100 nm compromise surface area to volume ratios, and has better interfacial and boundary qualities, which enable them to be superior in mechanical, thermal, and barrier properties. The below Table 1 shows the comparison of reinforcing improvement in NR products with various renewable organic reinforcing fillers. There have been several documented ways to alter the surface of natural fibers, such as grafting copolymerized monomers onto the fiber using maleic anhydride copolymers, alkyl succinic anhydride, stearic acid, etc. Additionally, coupling agents including silanes, titanates, zirconates, triazine compounds, etc. are known to enhance fiber-matrix adhesion.

Several research teams have previously looked at the applicability of jute fibers with and without surface modifications as fillers in rubber composites. The inclusion of jute fibers in rubber composites has been theorized as the cause of variance in key cure properties, including the maximum rheometric torque (R_{∞}), ideal cure time (t_{90}), and cure rate index. It was discovered that jute fibers treated with alkali/silane or modified with MWCNTs may both significantly increase the value of R for natural rubber composites. The correct dispersion of jute fibers inside the NR matrix and improved cross-linking in the NR composites are the key causes of this sort of enhancement in the R_{∞} value. The t_{90} and CRI values of composites were not significantly impacted by MWCNTs/jute fiber systems. On the other hand, an NR composite including 10 phr jute fibers and 2.5 phr stearic acid-modified nanoclay hybrid fillers system revealed an improvement in the CRI value, which demonstrated a positive synergism between jute fibers and stearic acid-modified system (Nair and Joseph 2014).

Hemp fibers were also employed as a green filler to strengthen rubber composites due to its high cellulose content (70%). Fiber and seed products made from different cannabis plant species are referred to as hemp. The usage of hemp strains cultivated

Table 1 Comparison of reinforcing improvement in NR products with various renewable organic reinforcing fillers

Reinforcing filler	Content	Tensile strength	Conclusion	Reference
Chitin nanocrystals (CNC)	20 wt%	116.4	The healing process of epoxidized NR/CNS's composites can be carried out in room temperature and can be used for sustainable material since CNS is a renewable material available in large quantity in nature	Nie et al. (2019)
Tunicate cellulose nanocrystals (one pot) Tunicate cellulose nanocrystals (two pot)	10 phr 10 phr	424.3 345.9	On comparing the result, it is seen that in both the composites hydrogen bond is formed and also the mechanical properties were increased	Cao et al. (2018)
Cellulose nanofibers (CNFs)	5 phr	35.4	CNFs are widely used as a reinforcing filler for rubber and resin; however, highly fibrillated CNFs are difficult to handle because it is hard to stop them from spreading. The use of CNFs in NR composites with the effect of their diameter distribution in mind should reduce the cost of production of CNFs, thereby enhancing their applicability	Kumagai et al. (2019)
Chitin nanofibers (ChNFs)	0.3 wt. %	145.3	This filler has high dispersibility and compatibility with natural rubber latex. They can be used for the making of artificial blood vessels, human diaphragm, etc.	Ding et al. (2019)

for low tetrahydrocannabinol (THC) content or with the THC eliminated otherwise is encouraged by legal restrictions for concentrations of the psychoactive drug components in hemp in many countries. Products made from refined hemp include fuel, hemp seed foods, hemp oil, wax, resin, rope, and textiles. The curing qualities of rubber compounds based on hemp fibers have received very little attention in past years. The integration of both surface-unmodified and surface-modified hem fibers resulted in a continuous rise in the magnitude of delta torque, or the difference between maximum and minimum torque. When compared to either unmodified HF's or silane-treated hemp fiber-based NR composites at the same filler loading, the improvement in the delta torque of potassium permanganate-treated hemp fiber-based NR composites was much superior. The way that water absorbs into a hydrophobic natural rubber matrix is less significant. The hydrophilic properties of hemp give its fibers a great propensity to absorb water. As a result, as the amount of fiber in the composites above grew, so did their water uptake percentage.

Additionally, the inclusion of hemp fibers in the composites complicated the process of water absorption in the rubber matrix (John et al. 2008; Krishna et al. 2022).

The seed of cotton plants belonging to the genus *Gossypium* develops in a bowl or protective capsule that is lined with a soft, fluffy staple fiber called cotton. Since this fiber is nearly entirely made of cellulose, cotton balls tend to spread the seeds more widely in their natural environment. The most popular plant fiber is cotton. Softness, good absorbency, color retention, and printability are just a few of cotton's qualities. It can also be machine washed and dry-cleaned, has good strength, drapes well, and is simple to handle and sew. It was effective to use cotton fibers to improve the mechanical qualities of NR composites. The addition of fibers has enhanced shear heating of composites due to the rise in bulk viscosity, which can also increase the rate of vulcanization. The optimal cure time of carbon fiber-reinforced composites decreased with an increase in the fibers loading. To increase the interfacial adhesion between natural rubber matrix and cotton fibers, maleated natural rubber is used as a coupling agent (Meissner and Rzymiski 2013). The amount of cotton used in natural composites consistently increased several significant mechanical parameters like hardness and tensile modulus, whereas the reverse tendency was shown for other mechanical properties like tensile strength and elongation at break. Due to the hydrogen bonding interaction between maleated natural rubber and the surface hydroxyl groups of cotton fibers, the aforementioned approach was able to significantly improve the mechanical characteristics of composites filled with cotton fibers.

Sugarcane bagasse fibers, both untreated and alkali treated, were utilized as filler for reinforcement in natural rubber. To untangle the web of hemicelluloses and lignin on the bagasse surface, alkali treatment proved quite helpful. The alkaline treatment has the potential to significantly shorten the composites' ideal cure period. In other words, as the percentage of alkali-treated sugarcane bagasse fibers used as filler in NR compounds increased, the optimal cure time reduced steadily. Composites made from natural fibers often have a wide range of uses in the building, construction, packaging, and automotive sectors. Natural fiber-based polymer composites have been widely employed by industrial businesses to create lightweight, ecologically friendly materials for the automobile sector. Natural fiber-based rubber composites have just recently begun to be used in the car sector. The fabrication of several interior components, including as seat bottoms, backrests, back cushions, and head restraints, has utilized a combination of natural rubber and coconut fibers. The primary use of natural fiber-based rubber composites in the car sector, however, remains debatable (Roy et al. 2021).

Cellulose Fiber-Reinforced Natural Rubber Composites

One of the most prevalent biomaterials on the planet is cellulose. Most often, plants generate it, but certain bacteria can also do it. Unlike starch, which has β -1,4 connections connecting its monomers, cellulose is a homopolymer of glucose. The sturdy formation of plant cell walls is greatly aided by cellulose, a hard, fibrous, and water-insoluble polysaccharide. The fibrils and microfibrils made up of cellulose

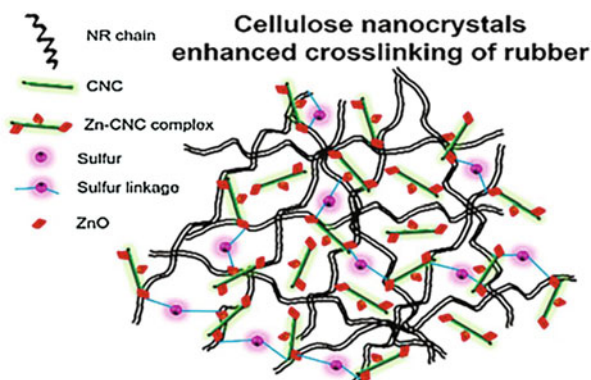
chains make up the plant cell wall. This arrangement shows that cellulose is a biomaterial with outstanding mechanical qualities and great strength, in addition to assisting in the stability of plant structures. Numerous living things, from the bacteria *Acetobacter xylinum* to forest trees, generate cellulose. The bacteria *A. xylinum* generates large amounts of cellulose, and it has been used as a model system for research on the structure of the cellulose product and cellulose production. The enzyme cellulose synthase, a membrane protein, catalyzes the direct polymerization of glucose from the substrate UDP-glucose into a cellulose product, which is how cellulose is produced (Nunes 2014).

These days, there are several techniques to synthesize cellulose at the nanoscale. Such nanofibrillated cellulose is normally quite strong and also possesses a number of intriguing qualities, including superhydrophilicity and intriguing rheological characteristics. Nanocelluloses can also be utilized in food, cosmetics, medicine, packaging, and many other applications. The projected usage is in novel lightweight composite materials for use in transportation and electrical applications. Cellulose-based NR composites demonstrate the combined behavior of the stiff, strong fiber reinforcement and the soft, elastic rubber matrix. The high amount of reinforcing available with long fibers is very difficult to achieve with short strands. However, because of their simple processing, high green strength, and the capacity to create complex-shaped products, short fiber composites are favored in goods like V-belts and hoses. Tensile strength develops a distinctive decline with increasing fiber volume content until a critical fiber level is achieved when fibers are oriented parallel to the stress direction (Phinyocheep 2014).

The maintenance of a high aspect ratio of the fiber, management of the fiber alignment, creation of a solid interface through physical-mechanical bonding, and formation of a high degree of dispersion can all improve the quality of a composite. In short elastomer composites, an aspect ratio of 100–200 is often needed for efficient reinforcing. Venugopal et al. found that adding carbon nanofibers to NR/cellulose nanofibers reduced cure time (t_{90}), but increased minimum and maximum torque (ML and MH) as the amount of added CNF increased. Furthermore, at 3 phr, the NR/CNF biocomposites showed outstanding NR reinforcement in terms of tensile strength, tear strength, hardness, and storage modulus. The diameter of reinforcing CNF affected NR; for example, the lower the diameter of reinforcing CNF, the greater the mechanical qualities of NR (Venugopal and Gopalakrishnan 2018).

Since both cellulose and NR are generated from renewable, biodegradable sources and are low-cost, low-density materials, using them together in composites has several benefits. Because they are simple to manufacture, have a high green strength, and can produce complex-shaped products, such composites are popular in goods like V-belts and hoses. These nanocomposite materials provide improved thermal, mechanical, and barrier capabilities at low reinforcement levels, as well as higher transparency and recyclability as compared to traditional composites. Results for NR/regenerated cellulose nanocomposites point to potential uses in regions where gas impermeability is crucial, as well as anytime the requisite mechanical performance of the article is better than that of a matching carbon black composition. Transparency, affordability, and biodegradability are other qualities that can be

Fig. 7 Illustration of formation of Zn-cellulose complex with CNC in the cross-linked natural rubber matrix (Blanchard et al. 2020)



necessary. Since they may be colored and have a light hue, NR/regenerated cellulose nanocomposites are appealing for industrial usage. Blanchard et al. investigate the addition of cellulose nanocrystals (CNC) to the NR matrix which increase the cross-linking effectiveness of the NR by forming a Zn cellulose complex, which improved ZnO dispersibility in the NR latex. The illustration of formation of Zn-cellulose complex with CNC in the cross-linked natural rubber matrix is illustrated in Fig. 7. The majority of applications are based only on laboratory research because there are few publications in the field of cellulosic nanofibers in NR matrix (Blanchard et al. 2020). Therefore, there is still a need to close the gap between research problems and commercial applications.

Marine-Based Natural Rubber Composites

The three primary physical spheres that make up the planet are air, water, and land. Water is essential for all life. The earth's surface is covered with water to a degree of around 80%. The aquaculture sector is expanding as a result of advances in aquaculture practices and rising human population. Consuming seafood at high and rising rates creates the issue of waste, or the components of marine food items that are not used. Waste from marine food items, particularly aquaculture animals, has been assessed for a variety of purposes because of its copious, affordable, and sustainable supply. Reusing discarded seafood is the most environmentally friendly approach to handle it (Thomas et al. 2021). Based on widely accessible, sustainably renewable agricultural and feedstock waste biomass, biodegradable plastics and bio-based polymer products do not ethically or financially compete with food production. Since marine animal shells cannot be consumed, they are a waste product from the food business and are not in competition with food demand, they are a low-cost commodity with no ethical issues.

The process of composite fabrication is such a way that the waste from acceptable marine products or marine crustaceans is first gathered. Typically, this comes in the

form of waste materials from the various food industries, but it can also take the shape of creatures caught by fishermen or gathered from the sea. The food sector views this as disposed as trash, creating an abundant supply of filler material. These discarded exoskeletons or shells are put through a washing procedure with fresh water for a number of washes before being dried in the sun to get rid of the remaining organic tissue and other impurities as well as the unpleasant smell. If NR is used, mixing the required quantity of marine powder, NR, and curing agent is the next stage in the rubber compounding process. A two-roll mill or internal mixer is used for mixing under previously experimentally adjusted settings (Boonrasri et al. 2020).

Inorganic components (CaCO_3 , SiO_2 , etc.) and biological components make up the majority of marine waste products (chitin, protein, etc.). The organic component of the biofiller acts as a compatibilizer to bind the filler particles to the rubbery matrix and increase the reinforcing mechanism. Due to their exceptional qualities, CaCO_3 -based compounds are now the most common engineering fillers used for industrial, medical, and environmental purposes. As a result, they make up more than half of this mineral's overall consumption. Additionally, using marine-based natural fillers like chitin (or chitosan, a derivative of chitin) from the aquaculture of crustaceans would assist to reduce waste and the need for nonrenewable petroleum and its derivatives. As a result, it will be easier to employ marine items as filler in NR vulcanizates. To determine what type of material may be used for industry, three requirements must first be met. First, the material needs to be able to sustain a supply while maintaining a competitive and steady price, which usually indicates there is not much competition from other uses. The second need is that the preparation procedure be simple, easy to manage, and eco-friendly. The materials must also be promptly available and require little to no modification (Poompradub 2014).

Chitin whiskers were used as the reinforcing phase and latex from both unvulcanized and prevulcanized natural rubber as the matrix to create nanocomposite composites. The chitin whiskers were created by Gopalan et al. (Gopalan Nair et al. 2003) by acid hydrolyzing the chitin from crab shells. Because unvulcanized evaporated samples include a chitin network, it has been found that they are more resistant to swelling tests than freeze-dried samples. Increasing the whisker content was also observed to reduce the level of sample swelling in each approach. The high swelling resistance against organic solvents was made possible by this stiff chitin network. Additionally, the relative weight loss, bound rubber content, and diffusion coefficient data were considered to validate the presence of chitin network. The resultant nanocomposite films' mechanical performance was examined in both the linear and nonlinear ranges. The capacity of chitin whiskers to create a solid three-dimensional network, brought on by strong interactions like hydrogen bonds between the whiskers, was crucial for the reinforcing effect. The findings from the subsequent tensile test studies provide convincing proof that the evaporated samples contain a three-dimensional chitin network.

According to A. Roy et al., the natural rubber/chitin composite's tensile modulus initially fell, then climbed as the chitin percentage rose until it reached its highest point. If more filler content is added after a material reaches its maximum tensile properties, reinforcing results in a reduction in the material's tensile strength. The

filler's inability to support the stresses imposed by the polymer matrix at lower filler levels can be used to explain the initial decline in tensile strength, and further increases can be attributed to the filler's ability to support the stresses imposed by the matrix as the filler quantity increases. The decrease in tensile strength following the ideal filler ratio demonstrates that as a material's reinforcement increases, its tensile strength also increases until more reinforcement is added (Roy [n.d.](#)).

Carbon Allotropes for High-Performance Natural Rubber

Rubbers are widely used in a variety of industries, including civil applications, biomedical applications, aerospace exploration, automotive engineering, and others. Although the unfilled rubbers often exhibit poor mechanical properties. In order to strengthen the rubbers, various fillers like as clay, silica, and carbon black must be used. These fillers make it simple to adjust the rubber's toughness, elongation at break, tensile strength, and modulus for a variety of applications. For the quick development of sophisticated technologies, rubber goods with some increased functionalities and exceptionally high mechanical performances are needed. Numerous interesting uses for 2D carbon-based materials exist, including biosensors, fuel cells, energy storage, and water filtration. New generations of sp² carbon allotropes have been accessible during the past few decades, including graphene, carbon nanotubes (both single- and multiwalled), and graphitic nanofillers consisting of a few layers of graphene. There are still many thrilling obstacles to overcome in the search for the rest of this family. There are many new allotropes to be created, and merging our fundamental low-dimensional forms into more intricate 3D buildings is extremely difficult (Fang et al. [2020](#)).

When contemplating the results of adding nanometer-scale particles, such as different carbon allotropes, to natural rubber, the phrase "reinforcement" can have a number of different interpretations. The element researched the most in the nano-age is carbon. Allotropes of carbon include graphite, diamond, fullerene, carbon nanotubes (CNTs), graphene (GE), and amorphous carbon, among others. These carbon structures have been studied for a number of years and are used in a variety of scientific and technological disciplines, particularly in rubber nanocomposites. A crucial contribution to the mechanical reinforcement is made by the nanostructured fillers CB and silica, whose aggregates include voids that can occlude and immobilize polymeric rubber chains. Cross-linked rubbers are typically brittle materials without additives. Depending on the size, shape, and type of elastomer-filler interactions, reinforcing filler particles can improve rubber's tensile strength, tear resistance, and abrasion resistance to variable degrees. They can also improve rubber's static and dynamic stiffness properties (George et al. [2021](#)). These all constitute enhancement of the mechanical properties.

Due to their improved qualities, carbon nanofillers and their nanohybrids reinforced NR and NR mix nanocomposites may find greater uses in tires, adhesives, surgical gloves, sealing materials, etc. Numerous researchers have examined the mechanical characteristics of various carbon filler-based natural rubber

nanocomposites. Carbon black is the most common type of filler (Robertson and Hardman 2021) used in demanding rubber applications, such as automotive tires and mechanical rubber goods. It comes in a variety of grades with a wide range of particle sizes (surface areas) and aggregate morphologies that are nanostructured to produce different property balances. The 15 million metric tonnes of carbon black produced annually across the world are utilized in rubber applications (73% for automotive tires and 20% for nontire rubber goods), with the remaining 7% going toward compounding paints, coatings, inks, and plastics. Fig. 8 shows the industrial applications of carbon black filler for demanding rubber applications.

The mechanical characteristics of multiwalled carbon nanotube–reinforced thermoplastic natural rubber nanocomposites were studied by Tarawneh et al. (2011). Using melt blending process polypropylene, liquid natural rubber, and natural rubber as compatibilizers with 1–7 wt% MWNTs were incorporated as nanocomposites. At 3 weight percent of MWNTs, the tensile strength and Young's modulus improved by about 39 and 30%, respectively. As the proportion of MWNTs increased, the elongation at break dropped. In comparison to a clean TPNR sample, the highest impact strength was measured at 5 weight percent MWNTs, a 74% increase. Hybrid nanomaterials have also been utilized to reinforce natural rubbers, including MWCNT/silica, MWCNT/CB, CNT-clay, and CNT-graphene. By using melt mixing procedures, Fritzsche et al. (2009). integrated MWCNTs in highly silica-loaded NR. The amount of silica has been sequentially replaced by the same amount of MWCNTs up to 10 phr of MWCNT in order to distinguish between attributes that are based on silica and MWCNT. The resultant samples exhibit improved tensile strength and mechanical stiffness. When a portion of the silica is replaced with 10 phr of MWCNT, the tensile strength rises from 14–17 MPa, however the elongation at break gradually decreases.

Carbon nanotube (CNT) is a nanofiller with considerable potential to enhance the characteristics of rubber composites, both single walled and multiwalled. In general, carbon allotrope's solubility parameter is determined by the functionalizing molecule while constructing the functionalization process. The carbon

Fig. 8 Industrial applications of carbon black filler as represented on the basis of reported data, adapted from reference (Robertson and Hardman 2021)



allotrope may be modified to be more or less compatible with various environments, including rubber phases. Functionalization techniques' major goal is to create dispersible tubes out of CNT bundles that can interact strongly with the matrix. Covalent or noncovalent functionalization, as well as endohedral or exohedral functionalization, are used to complete it. Rubber composites are regularly prepared using functionalized CNT. Utilizing oxidation methods, -OH and -COOH groups are preferentially introduced. Polar rubbers or rubber latexes are preferred when mixing CNT with polar groups. This composite offers higher mechanical and electrical characteristics, better qualities under stretching, decreased permeability to oil, etc. Stronger interfacial contact, also via covalent bonding, with rubber is achieved by using GO derivatives in the manufacture of rubber composites and by adding polar groups to the graphitic layers. Materials that self-assemble and heal self are produced when polar functional molecules, such as antioxidants, are loaded onto the filler (George et al. 2021).

The two techniques that are most frequently utilized to create rubber nanocomposites are melt mixing and solution approaches. Individual carbon-based fillers such CNT, CNF, graphene, expanded graphite, and graphene oxide exhibit improved mechanical and dynamical mechanical capabilities, which are explained by the state of dispersion of the fillers. The hybrid fillers made up of MWCNT or (CNF)-MMT, MWCNT-hectorite, MWCNT-LDH, and MWCNT-graphene in various rubber matrixes also significantly increase performance. These carbon-based fillers are assumed to have significantly increased tensile strength, impact strength, elongation at break, Young's modulus, stand loss modulus, storage modulus, and other properties of NR, SBR, NBR, and SR nanocomposites. However, more recent work must continue to be concentrated on the creation of related EPDM and EVA systems.

Natural Rubber for Asphalt and Bitumen Modification

The addition of polymers can improve asphalt mixtures' elasticity and flexibility, enhancing their resistance to thermal cracking and fatigue. The thermal and binding properties of natural rubber make it used in bitumen modification. It acts as an elastic band in the cold season and on increasing the temperature it functions as a film that improves the fracture toughness, thus preventing the movement of bitumen (Azahar 2016).

Raw NR latex is not utilized directly due to its high water content and sensitivity to bacterial attack. The latex must always be protected as well as concentrated by centrifugation, evaporation, and creaming so that the finished product is stable and includes 60% or more rubber. Usually, ammonia is added during these processes for preventing latex from bacterial attack (Bottier 2020). The introduction of NR to asphalt modification is accidental when there is a fall in concentrated NR latex price which is ready to sell in many top countries and this modification became a winning situation and big relief to the rubber farmers and also can improve the road transit system which are in poor shapes.

During the 1950s and 1960s, the majority of investigations on NR-modified asphalt were undertaken in the UK (United Kingdom). NR power (unvulcanized or moderately vulcanized) and NR latex were studied and compared. Adding NR powder to asphalt pavements had various benefits, along with improved rutting and fatigue resistance. However, it had some downsides, including a high mixing temperature and a long mixing period of up to 2 h (Wen et al. 2017). As the rubber particles in NR latex are well dispersed, they can be used to avoid such drawbacks. As an asphalt binder modifier, NR latex has several advantages, including better resistance to rutting and thermal cracking in asphalt pavements.

Natural Rubber Latex Composites

NR latex is a polymeric substance that is reusable and has outstanding physical characteristics and flexibility. Prevulcanized latex is an easy raw material for the production of thin film goods like gloves, condoms, and other medical items. To boost the film hardness and tensile strength, the rubber chains inside each NR particle must be treated with sulfur or partly cross-linked NR latex has some drawbacks, which includes low chemical resistance and processing capacity. Since prevulcanization causes the first cross-linking to occur within the latex particles, the physical characteristics of the finished product can be efficiently tailored before the articles are made. It is well known that the vulcanization systems affect how quickly the prevulcanization reaction occurs, and that the degree of prevulcanization has a significant impact on how the latex film forms. The cross-links between the rubber chains in the sulfur prevulcanized NR particle may be created by heating the combination of NR latex and the aqueous dispersions of S, ZnO, and accelerator zinc diethyldithiocarbamate. Other than prevulcanization, it has been claimed that chemical modifications of natural rubber latex can be used to get around certain inherent problems with NR vulcanizates caused by high unsaturation and the presence of allylic carbon, which results in changes in molecular structure. The corrosion and flame resistance offered by chlorinated NR latex can be used in coating formulation. Superior thermal stability is achieved by limiting oxidative degradation of NR as a result of hydrogenation and metathesis processes.

For the vast range of potential uses, such as paint, controlled-release medicine delivery systems, and toughening agents for thermoplastics, latex particles must have the appropriate characteristics and attributes. Due to its economic benefits and convenience in formulation, the modification of NR in latex state – a water-based, solvent-free, and low-volatile organic compound system – is preferred. To avoid interfering with their natural stability, creation of NR latex particles demands a deeper knowledge of their colloidal nature. The surface functionalization or hetero-phase radical polymerization process, which permits the insertion of functional groups or polymeric shell, can be used to modify NR particles in a manner similar to that used to modify synthetic polymer colloids. These manufactured particles with planned morphologies can be applied in a variety of contexts. For instance, when utilized as nanofiller, the modified NR particles give the films outstanding mechanical qualities as well as functionality. The presence of extensive double bonds in the

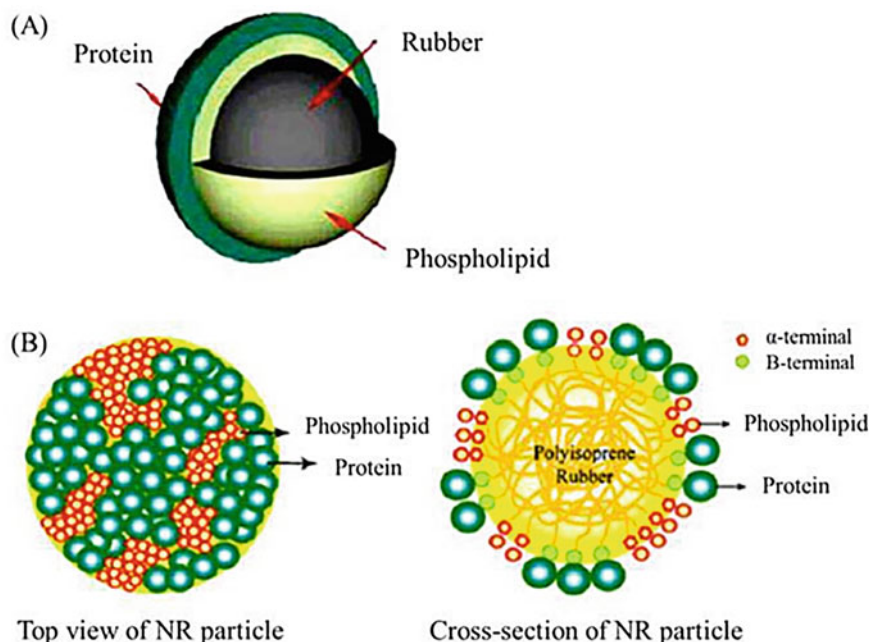


Fig. 9 (a) NR latex particle surrounded by double layer of proteins and phospholipids. (b) Proposed new model comprising a mixed layer of proteins and phospholipids around the latex particle (Nawamawat et al. 2011)

NR chemical structure accounts for the low resistance of NR latex thin films to heat, oxygen, and ozone (Kawahara et al. 2003).

Latex stability is extremely dependent on the pH and ionic environment in the dispersing medium. NR latex is colloidally stable due to the negatively charged phospholipid-protein complexes on its surface. The most abundant phospholipid is strongly attached lecithin on NR latex. Figure 5 shows that the negatively charged phospholipid protein complexes in the surface of rubber particle help in the colloidal stability of NR latex. According to Nawamawat et al., NR latex particle comprises a hydrophobic core of rubber molecules that are surrounded by a double layer of proteins and phospholipids Fig. 9a. The ω - and α -terminal ends of the NR molecule are connected with proteins and phospholipids, hydrophilic endings on the particle surface. The hydrophobic core should subsequently be formed by the polyisoprene molecules, resulting in a core-shell-like particle as shown in Fig. 9b (Nawamawat et al. 2011).

Epoxidized Natural Rubber Composites

Natural rubber can be epoxidized by the conversion of unsaturated linkages in rubber chains to oxiranes. Even though the first report about epoxidized natural rubber (ENR) happened at early 1920s, its commercial exploitation started only at late

1980s. The higher glass transition temperature compared to NR gives ENR some special properties like better damping, higher permeation to air, superior hysteresis, high wet grip, and, moreover, increased polarity. Epoxidation more than 20 mol% will not change the elasticity of original NR, but the presence of oxirane groups opens the possibility of a wide range of chemical reactions involving reinforcement systems and novel cross-linking systems.

Since the NR is nonpolar, the process of epoxidation increases NR's polarity and can also improve its chemical resistance and new properties are given to the polymer, commercially prepared using peracid. This process can elevate the oil resistance by maintaining the dynamic and static mechanical properties of NR. These composites are oil resistant in the same way as polar rubbers like nitrile butadiene rubber are. They have lower permeability toward nitrogen and oxygen and have good wet fraction and modulus compared with the natural rubber. Mainly used in the manufacture of tires and rubber mechanical goods (Gelling and Morrison 1985; Imbernon et al. 2016; Perera 1990), and also in the application of oil and abrasion resistance,

epoxidized natural rubber composites can be chemically modified by partially converting the unsaturated bonds on the backbone of NR to epoxy groups. Carbon black (CB)-filled composites are one such example that was made using epoxidized guayule natural rubber (EGNR)/guayule natural rubber (GNR) blends and GNR. It has been found that addition of ENR will definitely increase the impact strength of the resultant material. The load-bearing capacity was found to increase significantly as a result of rubbery phase morphology and extended chain conformations. Effect of ENR inclusion in varieties of blends and composites are summarized in the following Table 2.

The polar groups present in ENR is the key factor in increasing the toughening property of all these blends/composites as they form good interfacial adhesion. It was also found that the presence of ENR rubber phase is enhancing the uniform distribution of different types of fillers like OMMT and coal gangue powder. Introduction of ENR/LENR into epoxy systems also results in superior mechanical behavior. The toughness of epoxy system is enhanced due to the creation of discrete rubber particles in the precured epoxy mixture. Chemical cross-linking is formed between the two phases which include ring opening of epoxide and glycidyl groups by the carboxylic terminal in the rubber chains.

Applications of Gutta-Percha Rubber

Gutta-percha (GP), a revolutionary material, was first used in the early eighteenth century in several fields. In 1756, Europeans collected water-resistant shoes, textiles, and utensils from the Indian continent. Since working with impermeable, highly elastic gutta-percha is challenging, people began using these rubbers as adhesives at room temperature, especially when two pieces came into contact. When Alexander, Cbriot, and Duclos won the gutta-percha patent in 1864, it became an industrial product. Insulation of underwater cables, stoppers, pipe cement, threads, surgical equipment, clothing, musical instruments, suspenders, shutters, carpets, gloves,

Table 2 Effect of ENR inclusion in varieties of blends and composites. (Adapted from reference (Tanjung et al. 2015) with permission)

ENR/Polymer blend	Amount of ENR used (wt%)	Impact strength		Tensile strength (MPa)	
		With ENR	Without ENR	With ENR	Without ENR
PVC/L-ENR-20	10	900 J/m	310 J/m	35.60	38.50
PVC/H-ENR-20	10	850 J/m	310 J/m	32.00	38.50
PVC/ENR-50	50	—	—	15.90	38.00
PP/OMTT/ENR-25	20	118 J/m	24 J/m	—	—
PLA/ENR-50	10	7 MPa	4 MPa	59.00	80.00
PLA/talc/ENR-50	20	160 J/m	30 J/m	15.30	55.00
PA6/OMTT/ENR-50	10	5.8 kJ/m ²	2 kJ/m ²	51.00	84.00
PA6/HNT/ENR-50	10	7 kJ/m ²	3 kJ/m ²	56.30	85.00
Epoxy/Glass fiber/LENR	12	12.5 kJ/m ²	10 kJ/m ²	192.00	190.00
Epoxy-ENR-50	3.5	43 kJ/m ²	11 kJ/m ²	65.00	85.00
Epoxy/LENR-50	10	45.8 kJ/m ²	11 kJ/m ²	79.00	85.00

mattresses, pillows, tents, umbrellas, golf balls (gutties), ship sheathing, and boats made entirely of GP has all been successful (Goodman et al. 1974).

Gutta-Percha in Dentistry

In the 2000s, rubber-based dental dams for endodontic applications became increasingly popular. Reamers (hand instruments) were frequent material used for dental dams at that time which has been replaced by rubber, combined with sodium hypochlorite and EDTA as canal irrigants. Rubber-based dental dams and reamers showed no significant differences (Kapitán and Šustová 2011). These rubber-based materials were subsequently replaced by gutta-percha-based materials.

Although there is rigorous endodontic research occurring for better root canal fillers and preventive measures for microbial infections, gutta-percha is still one of the finest materials in dentistry even after 170 years. Gutta-percha has several advantages over other materials like low toxicity, less allergic, ease of use, and the flexibility to use as a matrix for a variety of fillers; also it is used to seal teeth canals. Evolution of gutta-percha in the field of dentistry is illustrated in Fig. 10. Modified gutta-percha based on bioceramic coating was used to enhance the quality of obturation, and reduce the shrinkage which can in turn benefit sealing the canals (Ahmad 2009). According to the adaptability of gutta-percha along with iodoform, calcium hydroxide, and chlorhexidine, chlorhexidine (CHX) and tetracycline enabled it to be applicable in different dentistry therapies which resulted in improved

1846- Alexander Cabriol surgical uses	1847- Edwin Truman-GP- Temporary filling material	1847- Hill-Hill's stopping restorative material- Mixture of bleached GP, carbonate of lime and silica [9]	1849- Chevalier, Poiseuille and Robert-GP tissue (laminated sheets)- Academy of Medicine, Paris
1864- First patent by Alexander, Cabriot, and Duclos	1867- Bowman-root filling material-St Louis Dental Society	1883- Perry-Softened GP with gold wire	1887- S. S. White Co.- Manufacture of GP
1893- William Herbt Rollins- Modified GP with Vermillion	1911- Webster-Heated GP- Sectional method of obturation	1914- Callahan-Softening and dissolution of GP	1959- Ingle and Levine- Standardized root canal instruments and materials
1959- Standardized GP - 2 nd International Conference of Endodontics at Philadelphia	1967- Schilder-Warm vertical compaction	1976- International standards organization group for approval of specification of endodontic instruments and materials	1977- Yee <i>et al.</i> -Injectable Thermoplasticized GP
1978- Ben Johnson- Carrier based GP-Thermafil	1979- McSpadden-Special Compactor-Softening of GP by frictional heat	1984- Michanowicz-Low temperature Injectable GP- Ultrafils	2006- ANSI/ADA specification-GP cones-No.78 ⁹ .

Fig. 10 Data obtained from Vijetha Vishwanath et al. (Vishwanath and Rao 2019)

antimicrobial properties, inhibiting the growth of *Staphylococcus aureus*, *Streptococcus sanguis*, *Actinomyces odontolyticus*, *Fusobacterium nucleatum*, *E. faecalis*, and *Candida albicans*, among other bacteria (Shur et al. 2003; Kim and Kim 2014; Öztan et al. 2006; Bodrumlu et al. 2008).

Evolution of Gutta-Percha in Dentistry

Gutta-percha is a biocompatible material that can be used for drug delivery, endodontic therapy, apical surgery, and rehabilitation. By incorporating nanoparticles into these biocomposites, they may be able to minimize root canal reinfection and improve treatment outcomes. Carbon nanoparticles, nanodiamonds (Lee et al. 2015), and silver nanoparticles (Shantiaee et al. 2011) have been used in the gutta-percha composite. Silver nanoparticles in gutta-percha have great antibacterial activity over a long period, are low in toxicity, have limited bacterial resistance, and are biocompatible with human cells.

The use of hot gutta-percha in thermal stimulation for measuring tooth vitality and intracorneal tooth preparation has become widespread. It can be accurate to 3 mm of the lesion in a diagnostic adjuvant in surgery. On the other hand, cold

flowable gutta-percha root canal obturation after apexification on young permanent teeth can obtain a good obturation outcome. When compared to hot gutta-percha, this cold flowable gutta-percha was found to be more effective in reducing clinical treatment time and improving patient therapy comfort. GP is inexpensive and readily available, its versatility and ease of usage with other materials have made it a popular choice for producing new materials for therapeutic purposes.

Gutta-Percha for Highly Stretchable Films/Membranes

Gutta-percha was started being utilized in the manufacture of coatings, packing materials, and other products replacing old petroleum-based materials, which polluted the environment significantly. It was employed as a biopolymer that is renewable and has good mechanical, thermal, corrosion, and electrical conductivity. It exhibits exceptional flexibility, with a reported elongation at break of 247.7% for the gutta-percha film, and is used as a material to be coated with compounds like zinc oxide and silver nanoparticles to increase the antimicrobial activity of the material (Mohan et al. 2020).

At normal temperature, gutta-percha can crystallize. Novel composites and products can be generated as a result of these individual qualities, or the properties of single-component biopolymers can be improved by mixing gutta-percha with these biopolymers. These biopolymer-based biofilms became very helpful in protecting soil coverings, packing materials, and crops against microbial inflation. Q.Q. Sun et. al found that biopolymers like nanocrystalline cellulose/gutta-percha have improved mechanical and water vapor barrier properties. Biochar was also used to develop GP-based biodegradable composite films that might be useful in a range of industries, including agricultural (mulching and pest protection or treatment), packaging, and electromagnetic interference shielding. This helps to replace potential pollution sources in the environment.

Gutta-Percha for Oil-Water Emulsions Separation

Gutta-percha is an isomer of natural rubber with the molecular structure of trans-1,4-polyisoprene. This structure gave some unique properties like enhanced hydrophobicity and rubber-plastic duality to GP. It is thought to be an excellent candidate for increasing nanofiber membrane flexibility and surface wettability. These renewable biopolymer membranes can be used as an alternative to petroleum-derived membranes.

Oil contamination of water has escalated into a significant environmental problem with long-term consequences for human health. To combat this oil contamination of water, the oil-water emulsifier should disperse. Separation of oil-water emulsions become difficult when the oil droplet diameter is lower to about 10–20 μm as the droplet gets more stabilized at that lower diameter range. Traditional chemical and physical processes such as flotation, absorption, and skimming can be used to

dispose off oily wastewater. However, those approaches are expensive, inefficient, and hard to implement, which is especially unfavorable for oil-water emulsions because the density difference has a significant impact on the separation processes. Thus, an alternative technology based on the wettability difference and sieving effect becomes popular in oil-water emulsions separation. Membranes for oil-water emulsions require these special wettability surfaces with suitable pore sizes allowing the wetting phase to penetrate the membrane. To ensure these requirements, membrane surface modification via an in situ blending or cross-linking can be done. Yichun Yahn et al. synthesized a blend membrane of silylated natural nanofibers and gutta-percha (Ren et al. 2018). This membrane was found to have excellent high hydrophobicity and crystallinity with good mechanical performance, thermal stability as well as water vapor permeability. Especially, the fabricated composite membranes exhibited excellent petroleum ether, dichloromethane, toluene, and cyclohexane in water emulsions separation effects. By integrating gutta-percha into silylated nanofibers from herbal leftovers, renewable hydrophobic membranes for oil wastewater (oil-water emulsion) separation have been successfully constructed. Instead of these silylated nanofibers, Sun. et.al used nanocellulose to improve the tensile characteristics of gutta-percha composite films (Yang et al. 2020).

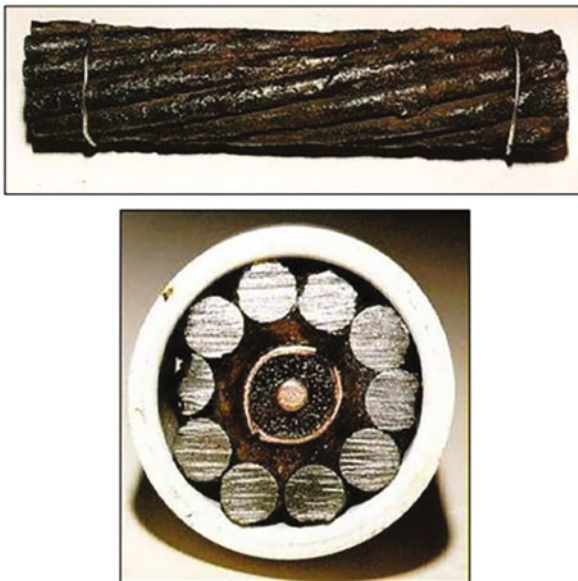
Gutta-Percha for Electrical Applications

Gutta-percha was made by evaporating the gutta-percha tree's milky fluid and coagulating the latex, yielding an inelastic solid insulator. In hot water, this material softened and gets hardened without becoming brittle after cooling. This is a unique advantage over Indian rubber, which had previously been used in cables. GP was designed to be used underwater, where submersion kept the wire out of sunshine which caused it to deteriorate and the cold and pressure environment enhanced its insulating properties.

Gutta-percha as an insulating material for electrical applications got its start in 1850 when a cable crossing the English Channel to France broke after carrying some signals. That cable was replaced by another cable brought from the Malay Peninsula which was coated with waterproof insulating material. It provided good inelastic, strong insulation that softened when exposed to hot water and cooled hard but not brittle. The main issue they had was that gutta-percha deteriorated in the sunshine (Faraday 1848).

Gutta-percha became an industrially prominent and easily processable low-cost material when it was initially used for electrical and water insulation for telegraph cables; various layers of gutta-percha-coated cables for electrical application are shown in Fig. 11. In the early 1800s, gutta-percha and balata were commonly used as underwater insulating materials. Long deep-sea telegraph cables have too high dielectric losses to suit the demands of underwater communication system cables that must operate over long distances or shorter cables that use carrier currents.

Fig. 11 Various layers of electrical cables coated with gutta-percha. (Adapted Reference Panuganti et al. 2016)



To deal with the mechanical instability of gutta-percha or to adjust its mechanical properties, a new material called paragutta was developed. Pure balata (gutta-percha) hydrocarbons and rubber hybrids make up the majority of it, with a few waxes tossed in for good measure. This commercial method was used to purify rubber, particularly in terms of nitrogenous components, which was necessary for enhanced electrical stability in water. Paragutta had all of the desirable features of thermoplastics and gutta-percha's mechanical properties, as well as improved insulating properties. Its suitability for both long-range transoceanic and shorter-range coastal maritime telephony was improved as a result (Kemp 1931).

Conclusion

After air, water, and petroleum, natural rubber is the fourth most significant natural resource on the modern earth. Due to its distinctive molecular identities, NR is predicted to be used more and in higher-quality products throughout this century. Additionally, the twenty-first-century global environment will require particular types of renewable resources if sustainable development is to be implemented in daily life. The natural rubber-based nanocomposites have a wide variety of application in many fields and the replacement of synthetic filler with bio-based filler increases its application in many areas and also increases fiber content leading to an

increase in the strength and modulus of a fiber-reinforced rubber matrix composites. Although some dentists still use it to create temporary fillings, gutta-percha is rarely utilized any more. It is occasionally combined with balata resin, a very comparable substance used in conveyor belts. To use natural rubber and gutta-percha more methodically and following its characteristics, research- and application-focused initiatives have to be activated.

Government laws, customer preferences, and in certain cases the potential financial benefits that may be gained from the use of these materials and technologies have all contributed to the industry's increasing emphasis on environmentally friendly materials and technology. Bio-based composites have undergone years of development, and they are now getting closer to matching or even surpassing the cost and performance criteria of conventional composites. However, despite these developments, there are still several issues that limit the use of bio-based products in the automobile sector. Automakers are typically hesitant to pay a premium for parts and components due to the fierce pricing competitiveness in this industry. Therefore, suppliers need to overcome this flaw in bio-based products.

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Samuel Ogbeide Ebhodaghe

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Abstract

Albumin is one of the most abundant plasma proteins found in recent times to possess multifunctionality. This chapter reveals the properties and engineering applications of albumin-based hydrogels influenced by chemical crosslinking, drug-coating techniques, pH, and thermal induction. To provide context, we first examine the nanotechnological techniques of developing albumin nanoparticles. Though albumin hydrogels generally find application in tissue engineering, they could also find usefulness for skin and wound healing, as well as toxicological studies involving stem cell-derived nerve cells, disease modeling, and drug delivery, when chemically cross-linked. This is because, cell survival and growth of

S. O. Ebhodaghe (✉)

Chemical Engineering Department, University of Benin, Benin City, Nigeria

e-mail: samuel.ebhodaghe@eng.uniben.edu

chemically cross-linked albumin is possible, rather than when subjected to severe heat and acidic or alkaline conditions. Finally, albumin coatings via linkage engineering, electrostatic interactions, and electrostatic deposition are presented.

Keywords

Albumin hydrogels · Nanotechnology · Delivery systems · Engineering applications · Properties

Introduction

Albumin is an ubiquitous plasma serum protein presently investigated for several applications, because of its multifunctionality (Ong et al. 2019). Specifically, albumin-based hydrogels are researched for applications in tissue engineering and regenerative medicine since they are inert (Ahmed 2015; Elsadek and Kratz 2012), nonsynthetic, xeno-free, stable, and nontoxic (Das et al. 2012; Yamasaki and Anraku 2016) with a molecular weight of 66.4 KDa, and having about 585 amino acids (Dixon et al. 2020). Albumin-based hydrogel composites are widely explored due to their desirable mechanical and biological properties (Clura et al. 2020). This binding with other composite materials becomes necessary to address the present challenges of poor metabolism, poor immunogenicity, and other limitations associated with albumin protein (Solanki et al. 2021; Haggag et al. 2018).

The improved mechanical properties of albumin when induced thermally and chemically cross-linked include Young's modulus of -46 Kpa, and -34 Kpa for 20 wt% (Amdursky et al. 2018a), and 17 wt%, bovine serum albumin (BSA) (Baler et al. 2014), respectively. Also, tensile strength of -2 – 5 Mpa for 3–9 wt% BSA, and -40 MPa for 10 wt% human serum albumin (HSA) (Zhao et al. 2019; Khairuddin et al. 2018). In addition, most albumin hydrogels are printable under thermal conditions since heat is required to induce gelation (Djoullah et al. 2018). However, the coatings with other materials, particularly drugs, enhance the half-life and treatment efficiency of the drugs (Park et al. 2018) and improve albumin biocompatibility, biodegradability, immunogenicity, metabolism, hydrogel turbidity, storage, and loss modulus, amid others (Naftaly et al. 2021; Arabi et al. 2020).

Presently, more studies are emphasizing novel techniques of enhancing several properties of albumin composites than their applications. Within the last 6 months, numerous studies have emphasized properties improvement. To illustrate, Naser et al. (2022) report on the need to enhance the biodegradable nature of albumin-based composites for suture development in tissue engineering. Also, Wang et al. (2022a) argue that color tunability and self-healing properties of albumin hydrogels should be addressed through a reductant-triggered gelation. They believe that when developed, this protein-based hydrogel could be used to develop optical devices and enhance biomedical applications. Similarly, Sharifi et al. (2022) report that HSA could be a suitable platform for different biomedical applications when the anti-inflammatory properties of the hydrogel are improved through a methacryloylated

synthesis process. Few studies that have considered direct application of albumin-based composites have reported on their use for delamination of MXenes for application in environmental engineering, medicine and biology (Seredych et al. 2022), therapeutic applications with fatty acids interactions (Linciano et al. 2022), and for electrical stimulation of heart cell growth (Gerasimenko et al. 2022).

These applications are possible because albumin binds with other materials including carbon nanotubes (Lu et al. 2018), curcumin-capped mesoporous silica nanoparticles (NPs) (Fu et al. 2022). Research have shown that albumin-based NPs are most commonly used for various applications. In this chapter, we present nanotechnological techniques for producing albumin NPs and consider albumin-based hydrogel applications in drug delivery and clinical conditions.

Albumin-Based Hydrogels for Regenerative Engineering: Synthesis and Properties

For pH-induced albumin hydrogels, it is possible to obtain a clear hydrogel polymer from soluble albumin by altering the pH conditions. Studies have shown that reducing the pH below 2.3 changes albumin structure to an extended form. Similar pH changes between 2.3–4.2, 4.3–8.0, and 8–10 adjust the hydrogel structure to fast migrating, normal, and basic forms, respectively. Albumin could also become aged when pH is adjusted beyond 10 (Ong et al. 2019; Baler et al. 2014). On the mechanical properties, Baler et al. report a Young's modulus of 46 kPa for 20 wt % BSA via indentation. Also, 5–10 and 60–80 kPa storage, G' and loss modulus G'' , respectively, were noted for the 16 and 20 wt% BSA after 2300 and 340 s, respectively. The hydrogels exhibit low immunogenicity, rapid biodegradability, difficult printability, and “clear to translucent hydrogel turbidity.”

Considering thermally induced albumin hydrogels, studies have shown that heat-induced gelation process can be used to ensure stability of albumin hydrogels (Nandlall et al. 2010; Lantigua et al. 2020). Young's modulus of the hydrogels is ranged about 34 and 67 KPa at a pH condition of 3.5 for 17 and 20 wt% BSA hydrogels (Amdursky et al. 2018b). Under conditions of tension and confined compression, the Young's modulus differs even for same weight BSA hydrogels. Though huge amounts of heat are required, printability is possible, low immunogenic effect is observed, and biodegradability occurs over a month. Notably, the storage G' and loss modulus G'' were 120 and 60 KPa for both 16 and 20 wt% hydrogels, respectively. Recently, Lantigua et al. fabricated BSA hydrogels through a photo-cross-linking method with volumes of glycidyl methacrylate in the modification process. Based on the study, the BSA can be used in regenerative medicine and other biomedical applications. The hydrogels resemble those of native tissues due to tissue infiltration in the host tissue, and high binding ability to the native molecules. This illustrates the extent of biocompatibility and integration with the tissue environment. Similarly, the hydrogel materials have desired ranges of mechanically tunable properties including strength of the materials, pore sizes, swelling, and degradation.

More recently, Phan et al. fabricated and applied the triblock copolymer of poly (E-caprolactone-co-lactide)-b-poly(ethylene glycol)-b-poly(E-caprolactone-co-lactide) with BSA bioconjugates for wound healing. Despite the wound healing effects of these injectable hydrogels, they could also be used in regenerative medicine when correctly modified. Biocompatibility of the gels was demonstrated via the fact that “the tissue was intact without any inflammation” according to the study. Also, since improved tissue-like mechanical properties are required for its repair and regeneration (Ebhodaghe 2020, 2021), presence of the “protein-polymer bioconjugates” aided tunability of the mechanical properties of the hydrogels (Chen et al. 2020). Furthermore, Smith et al. (2020) developed the protein constructs of 3D – printable methacrylated BSA through a two-step additive-manufacturing process using a laser-based stereo-lithography printer. These hydrogels can be used for load-bearing in tissue-engineering applications. This is because, cell viability of the hydrogel (>95%) was observed after 3 weeks of culture.

Influence of Cross-Linking and Thermal Induction on Albumin

Amighi et al. (2020) examined the possible use and effect of different cross-linkers (such as tannic acid, glucose, citric acid, ascorbic acid, and sorbitol) on albumin prepared through desolvation process. Overall, the use of glucose cross-linking agent was preferred to toxic glutaraldehyde. This is because, the parameters of size, zeta potential, and FTIR were similar for both cross-linking agents. Moreover, their physical characteristics were same.

On thermal effect, Zhao et al. (2021) prepared doxorubicin (DOX)-based hydrogel through a molecular imprinting method. The DOX imprinted hydrogel exhibited “much higher affinity and selectivity to DOX” than the albumin. This became possible through processes of thermal denaturation, refolding of the thermally denatured BSA, and then polymerization and cross-linking with formaldehyde. Also, Matsarskaia et al. (2020) investigated the influence of common salt on the statics and dynamics of thermally denatured BSA, examined via small-angle neutron scattering and quasi-elastic neutron scattering. While the salt had no obvious effect on the stability and dynamics of the denatured solution, the protein dynamics slowed down in the salty solution. Studies have similarly reported the influence of amino acids (glycine, alanine, proline, and arginine) on the thermal stability and unfolding of BSA (Pal et al. 2020).

Techniques for Albumin Production

Albumin-Bound Technology

Modification of albumin enables its usefulness in specific organ and cancer targeting (Lamichhane and Lee 2020). While a general overview of nanotechnological techniques for albumin modification is presented in Fig. 1, specific modification for target delivery to tumor and cancer sites is shown in Fig. 2. For

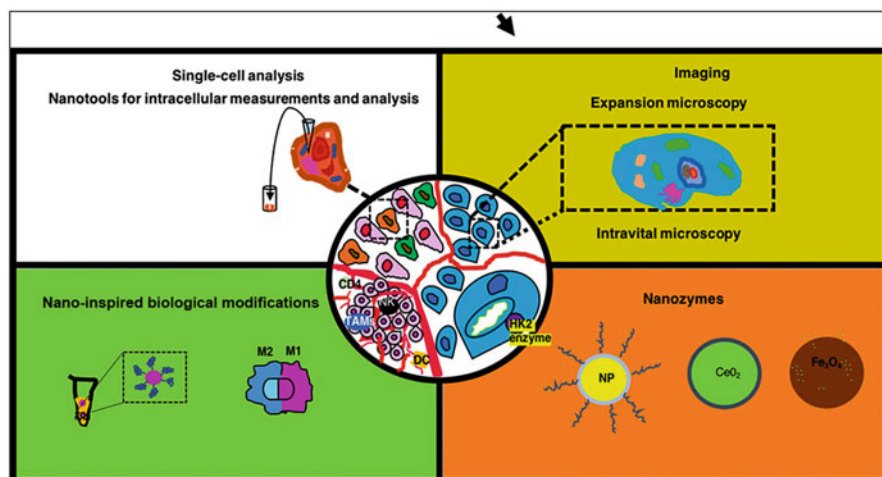


Fig. 1 Nanotechnological tools for albumin modification (Reproduced with permission from: Carolina Salvador-Morales, Piotr Grodzinski. *ACS Nano* 2022, 16, 4, 5062–5084)

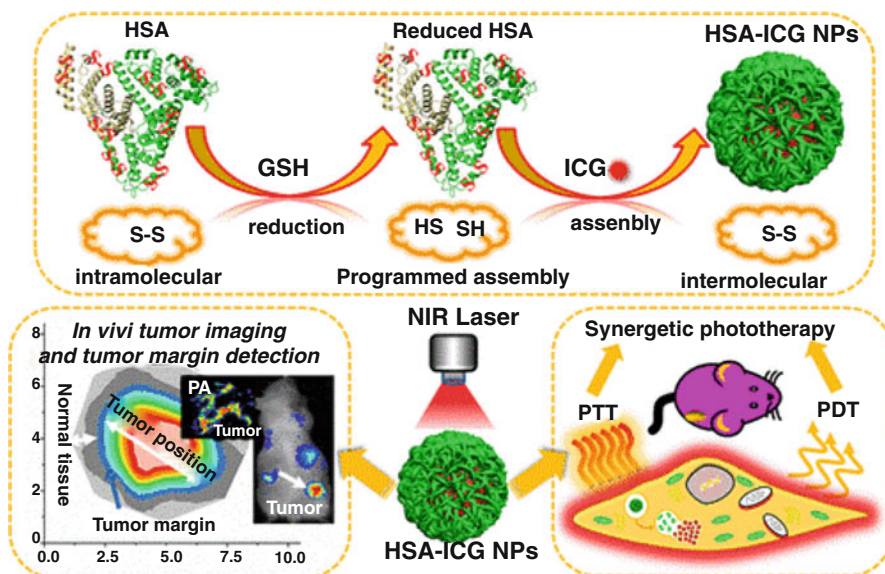


Fig. 2 Albumin nanoparticles for cancer and tumor phototherapy in rodent models (Reproduced with permission from: Zonghai Sheng, Dehong Hu, Mingbin Zheng et al. *ACS Nano* 2014, 8, 12, 12310–12322)

example, several techniques have been developed for liver as a target site which includes targeting asialoglycoprotein receptors (Lamichhane and Lee 2020), targeting mannose-6-phosphate/insulin-like growth factor 11 (Feczko et al.

2019), targeting mannose-6-phosphate receptors (Yang et al. 2018), and targeting glycyrrhetic acid receptor (He et al. 2018). Notably, doxorubicin (DOX) -based recombinant human serum albumin (rHSA) incorporated with glycyrrhetic acid targeted to liver cancer cells. Besides recombinant HSA, DOX could be also loaded with BSA nanoparticles (NPs) with folic acid-modified dextran surface (Lee et al. 2020), BSA/chitosan core (Shen et al. 2018), PLGA NPs (Cao et al. 2019), Fe₃O₄ magnetic NPs (Norouzi et al. 2020), and hyaluronic acid NPs (Tian et al. 2019). Also, the HSA NPs could be loaded into loperamide for targeted delivery to the brain (Harti et al. 2021).

When HSA is compared to Fe and PEG drug delivery vehicles, though these are all very soluble in water, only HSA and Fe are quite biocompatible in their low immunogenicity and toxicity within few weeks, and they are also biodegradable (Juan et al. 2019; Levy et al. 2015). However, only HSA is more suitable for drug delivery. This is because of several reasons. First, it exhibits higher loading efficiency which makes it able to bind metal ions, synthetic drugs, peptide hormones, and proteins (Saleh et al. 2019). Next, the HSA is more stable than the others (Qu et al. 2019). This is due to its ability to resist high temperature conditions as well as low pH (Poureshahi et al. 2017). Finally, it is more tumor specific, which is due to SPARC and gp60 binding capacity (Li et al. 2017). Figure 3 shows how these protein pathways could aid drug delivery to target sites.

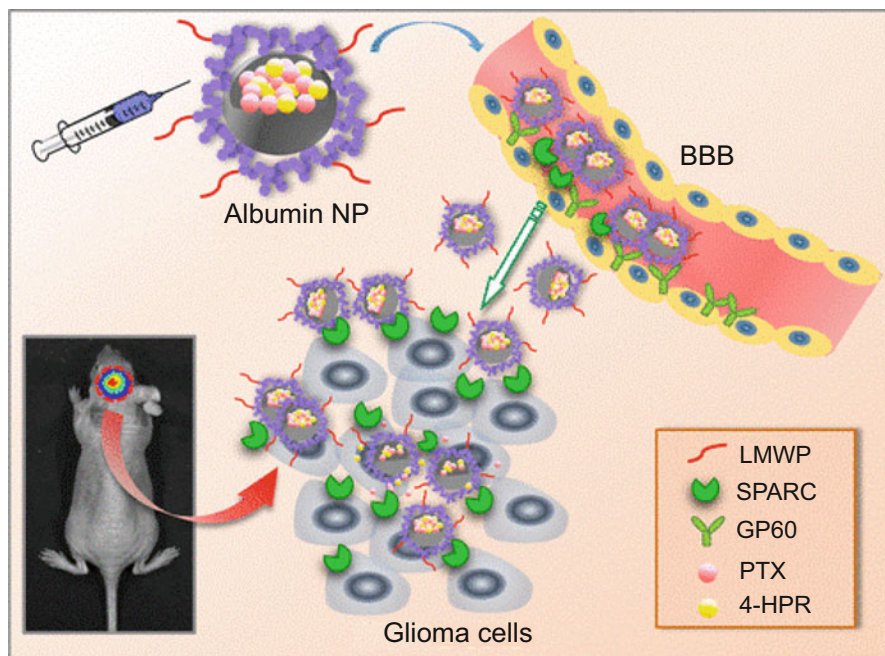


Fig. 3 Albumin-based biopolymers for drug delivery to targeted sites via protein pathways (Reproduced with permission from: Tingting Lin, Pengfei Zhao, Yifan Jiang et al. *ACS Nano* 2016, 10, 11, 9999–10012)

Self-Assembly

Presently, while novel techniques for preparing albumin nanoparticles for enhanced delivery are underway, the need to improve upon their properties of solubility and antimicrobial activities is now investigated. Yu et al. (2022a) prepared albumin NPs using *Camellia* seed cake (CSC) waste via the new technique of Self-Assembly. This method could also be used to develop other NPs, such as those of *Stauntonia brachyanthera* seed albumin, gum Arabic, and carboxymethylcellulose (Poureshahi et al. 2017; Li et al. 2017; Yu et al. 2022a), which are essential to enclose lutein. This is because lutein has enhanced stability and bioaccessibility after loading with the NPs. From the earlier study, the CSC-based albumin incorporated with chitosan was prepared, as the presence of the albumin increased the stable and bioaccessible nature of lutein for its delivery (Yu et al. 2022b). Overall, Self-Assembly could be seen as a simple, easy, and cost-effective means to fabricate the NPs (Li et al. 2021; Lian et al. 2017). Furthermore, Salih et al. (2021) developed polyethylene glycol-fusidic acid for self-assembling into the NPs. This was needed to improve the aqueous solubility, antibacterial action, as well as reduce the serum albumin binding of fusidic acid (Dziwornu et al. 2019). In brief, this PEG-FA made FA more soluble and antibacterial.

Emulsification

The recent study of Yang et al. (2022) shows that emulsion-stabilizing properties of albumin were similar to those of other proteins such as vicilin and legumin, which are present in Bambaran groundnut. The study had several findings about the albumin. First, they could form viscoelastic solid-like air-water interfaces with high dilatational moduli up to 9 N/m. Also, they displayed strong interfaces because of the presence of albumins. So, the albumin-stabilizing foams were almost same as those of whey protein-stabilized foams (Djemaoune et al. 2019; Pycia et al. 2017).

Coacervation

More recently, Zhang et al. (2021) examined the reaction between ovalbumin and pectin, which depends on temperature and pH. This association influenced their complexation, and a rigid layer formed in-between them. The presence of pectin introduced changes into the structure of ovalbumin. Moreover, the ovalbumin was thermally stable in the coacervation. While pH influenced the characterization, pH (12-1) and ratios (Ong et al. 2019; Haggag et al. 2018) have been reported to determine turbidity changes of scallop *Patinoplectin yessoensis* male gonad hydrolysates and k-carrageenan/xanthan gum. As such, they display well-organized and compact networks (Yan et al. 2022). At pH of 6.0–6.5, Kornet et al. (2022) show that solutions of processed pea protein displayed coacervation and the internal protein content of the coacervates was about 45 wt% at pH of 6.25. These findings were reported in absence of albumin.

Albumins are usually more soluble at pH 3–9 with higher foaming capacity (Ajibola et al. 2016) than others such as globulin and protein concentrate.

Thermal-Induced Aggregation

Rondeau et al. (2010) have presented spectroscopic studies on thermal aggregation of native and engineered BSA having different glucose concentrations. Changes in the secondary configuration of the BS aggregates were examined by Fourier Transformed infrared Spectroscopic absorption, while those of its tertiary structure were studied using fluorescence spectroscopy. The study shows that glycation-based changes in BSA affect aggregation process. Notably, the continuous increase of glucose concentration incubated with BSA hinders aggregates formation. More recently, Chandel et al. (2019) investigate the “molecular basis of the inhibition and disaggregation of thermally induced amyloid fibrils of human Serum albumin by an anti-Parkinson’s drug, benserazide hydrochloride.” Also, Zakariya et al. (2022) have shown that Sunset Yellow could restrict the activities of glycated thermally induced HSA aggregates in naturopathy.

Albumin-Based Hydrogel Applications

Albumin in Tissue Engineering

In cardiac tissue engineering, Amdursky et al. (2018b) have developed elastic serum-albumin from low-weight hydrogels as substrates for cardiac cells in vitro. As such, the hydrogel was used as a biomaterial for several other cell types, since elasticity of both BSA and myocardium were similar. A similar study considered the conversion of albumin into an available biomaterial with its cell compatibility (Li et al. 2014). This is important because albumin is a source of autogenic biomaterial for autologous tissue engineering. This tissue scaffold can be used for different tissue engineering applications, and it could also advance artificial fabrication of autogenic tissue substitutes. This is because, the material has a porous structure, and moderate mechanical strength resilience. Also, there is close compatibility with cells, as well as material properties. A more recent study examined the preparation of viable cell clusters of hepatocytes from Hep-G2 in large quantities. This is responsible for the presence of water in water emulsion cell-clustering platform in tissue regeneration (Wang et al. 2022b).

Albumin in Drug Delivery

HSA plays several roles in drug delivery to different sites/systems in the body. Recent studies show that the most common roles of the HSA are in the controlled and sustained release of paclitaxel, docetaxel, and doxorubicin-based drugs for

treatment of sarcoma, Type 2 diabetes, and prostate/breast/lung/ovarian cancers (Gao et al. 2019). The use of exogenous HSA-drug conjugation for these deliveries is commonly reported. Moreover, HSA is similarly used in tumor localization of several drugs including docetaxel N- E-maleimidocaproic acid hydrazide HSA, DOX COL-binding domain HSA, paclitaxel, aldorubicin, and others (Cranmer 2019; Jacob et al. 2018). Other roles of the albumin-based drug delivery systems are in targeted delivery to prostate and colon cancer and types of diabetes through improved PKWY, and higher cellular uptake (National Cancer Institute; De Groot et al. 2019). In brief, albumin binds to drugs through several techniques such as conjugation, in situ binding, genetic fusion, and NPs.

Albumin Coating Techniques for Clinical Applications

Albumin can coat synthetic compounds and NPs through several methods in varied clinical applications. This coating is a process of binding to compounds through several routes, which brings about reduced nonspecific protein adsorption and improved cell adhesion and proliferation (Zink et al. 2016; Kobayashi et al. 2017). Other routes different from protein pathways are shown in Fig. 4.

Albumin binds onto polyethylene and polyethersulfone through a poly dopamine layer which is an antithrombotic coating to resist protein fouling and enhance

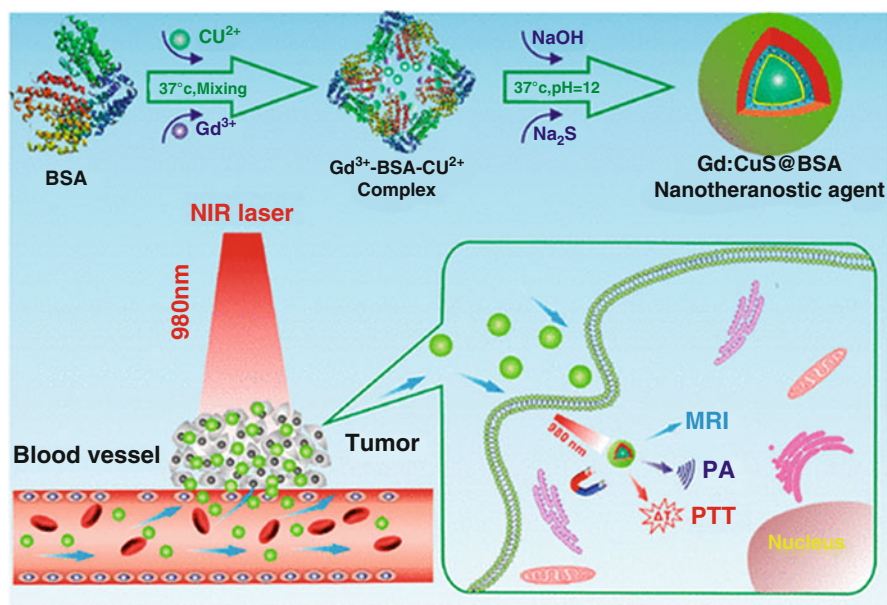


Fig. 4 Albumin nanoparticles for photothermal therapy in rodent models (Reproduced with permission from: Weitao Yang, Weisheng Guo, Wenjun Le et al. *ACS Nano* 2016, 10, 11, 10245–10257)

hemocompatibility (Sandeman et al. 2017). It is possible to reduce the nonspecific adsorption of proteins in albumin when it has been used to coat poly-hydroxyethylmethacrylate, polyurethane, and glass through affinity with octadecyl isocyanate and its reaction with epoxides on epoxysilanated glass (Tao et al. 2019). Improving the proliferation of mesencymal stem cells and antimicrobial action becomes realizable when albumin incubates demineralized bone matrices and polystyrene in protein solution at around 4 °C for an extended length of time (Pinho et al. 2021; Haag et al. 2022).

For albumin coatings on specific NPs such as liposomes, DNA origami, and tobacco mosaic virus, the possibility of attenuating immune response and prolonging blood circulation time becomes real (Achilli et al. 2022; Prabha and Raj 2017). These albumin coatings could be reached via engineering by linkers and reaction between maleimide groups and thiols for the tobacco mosaic virus, cysteine-maleimide bond, and electrostatic interaction for the DNA origami and affinity with ligands for liposomes (dos Santos et al. 2017; Ebhodaghe 2020). Other binding techniques of electrostatic interactions and electrostatic deposition are utilized for synthetic polymers such as poly (hydroxybutyrate-co-hydroxyhexanoate) and poly (methymethacrylate), respectively (Ebhodaghe 2021; Ma et al. 2017).

Conclusion

We have reported the use of albumin-based composites for several drug delivery and clinical applications. Several mechanical and biological properties enhances their disease-targeting “in molecular cancer therapeutics.” Also, we have seen that albumin-based composite coatings could be obtained when they are engineered by linkers, other reactions, and binding techniques.

Cross-References

- [Applications of Biopolymer-Based Nanofibers and Nanomembranes](#)
- [Biopolymer-Based Composites](#)
- [Mechanical Properties of Biopolymers](#)
- [Nature-Inspired Biomimetic Polymeric Materials and Their Applications](#)

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Tunable Biopolymers

30

Biomedical Applications

Amee Krishnakumar, Urja Shedaliya, Kavya Shah, and T. R. Anju

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A. Krishnakumar (✉) · U. Shedaliya · K. Shah
Institute of Science, Nirma University, Ahmedabad, Gujarat, India
e-mail: ameenair@nirmauni.ac.in; 21ftphds63@nirmauni.ac.in; 21ftphds65@nirmauni.ac.in

T. R. Anju
Department of Biotechnology, Newman College, Thodupuzha, Kerala, India
e-mail: anju.tr@newmancollege.ac.in

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Abstract

Since the turn of the century, biopolymer uses in the biomedical field have seen a tremendous rise owing to their abundance, biocompatibility, efficacy, minimal immunogenicity, and biodegradability. Biopolymer-based scaffolds - 2D films, hollow fibers, hydrogels, sponge, 2D/3D Electrospun fibers, microcarrier beads are used to produce prototypes to tackle many obstacles in the fields of biotechnology, nanoscience, and in vitro investigations for TE including bone, neuron, muscle, tendon/ligament regeneration. Besides electrospinning nanofibers as well as 3D bioprinting have sparked a lot of consideration as a forthcoming scaffold for the application in the field of TE and wound dressing. Biopolymers have piqued researchers' interest in determining the efficacy of drug delivery to a specific region employing them as a carrier. Wound healing applications are now heavily reliant on biopolymers due to their tunability aiding their employment in production of wide-range of efficient healing-enabling products ranging from woven fibers that expands on exposure to exudate to water-rich hydrogels that prevents drying of wounds. Post-surgical physiological functioning as well as progressive diseases can be monitored using biodegradable biopolymer-based biosensors. The disintegration of the employed biopolymer-based implants reduces the need for a surgical retraction of graft as well as graft rejection. There are an almost endless number of physical behaviors that can be modified for a given purpose due to the large range of biopolymers available and the ability to combine as biopolymers, some of which are discussed later in this chapter.

Keywords

Bio-polymer · Bio-sensors · Bio-medical implants · 3D-Bioprinting · Hemostatic agents · Hydrogels · Microcarrier beads · Drug-delivery system

Introduction

In the recent years biopolymers have garnered growing importance in our daily life due to the tremendous rise in their applications especially in the field of pharmaceutical and medical sciences. Its abundance, bio-compatibility, effectiveness, relatively economical, low immunogenicity, and bio-degradable properties are the versatile traits which have allowed its wider acceptance and utility in biomedical therapeutics. These polymeric biomolecules are derived from living organisms including plants, animals, and microbes. Compared with their synthetic counterparts, these biopolymers are known to induce less toxicity, antigenicity, and immunological consequences. Biopolymers applied in the field of biomedicines are as follows (Figs. 1–2).

Biopolymers Application in Biomedical Sciences

Biopolymers and Their Attributes

Biopolymers consist of repeated monomeric biomolecule units which in fact define their property. Polynucleotides contain nucleotide repeats, polypeptides are formed of amino acids whereas polysaccharides are formed of repeating monomers of monosaccharides (Glieder et al. 2015). A few of the frequently used bio-polymer having bio-medical application are tabulated as below (Figs. 1–2).

Scaffolds and Matrices Employed in Bio-engineering

Scaffold is basically a material specifically fabricated to provide a platform which may be used in regenerative tissue engineering (TE), drug delivery, and

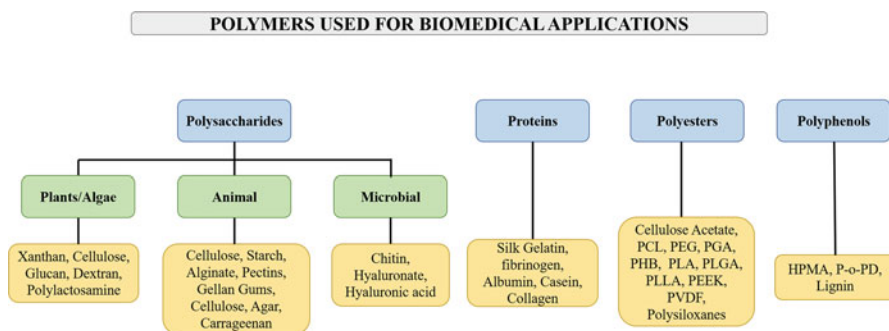


Fig. 1 Biopolymers derived from plant, animal and microbial sources which have applications in bio-medical fields. Polycaprolactone (PCL), Polyethylene glycol (PEG), Poly-Glycolic Acid (PGA), Polyurethanes (PUs), Poly (lactic acid-co-glycolic acid) (PLGA), Polyethylene vinyl acetate (PEVA), N-(2-hydroxypropyl)meth-acrylamide (HPMA) copolymers, Poly(L-lactic acid) (PLLA), Polyvinylidene fluoride (PVDF), Polyetheretherketone (PEEK), Polyhydroxy butyrate (PHB)

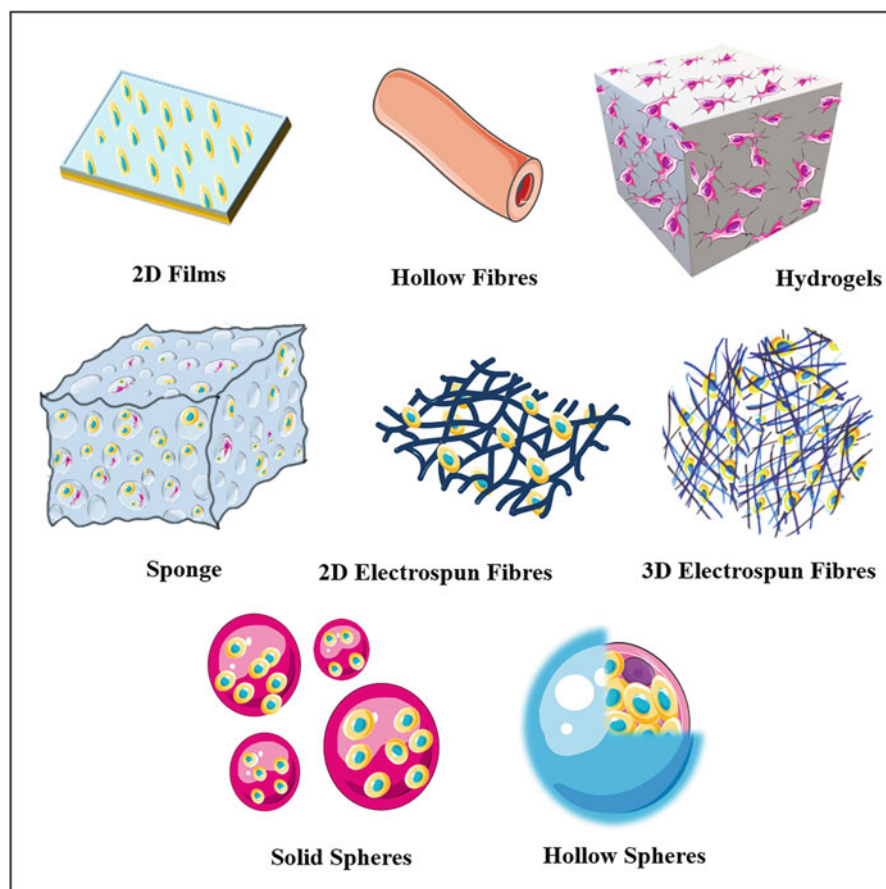


Fig. 2 Various geometries of biopolymer scaffolds and matrices employed in biomedical fields

immunomodulation. Scaffolds are known to repair the damaged or lost organ or tissues in TE (Haider et al. 2020). Biopolymer-based scaffolds and matrices are being used in various forms such as 2D films, hollow fibers, hydrogels, sponge, 2D and 3D Electrospun fibers, and solid as well as hollow spheres. TE has been an ever-evolving science which aims to use cells, scaffolds and growth factors under *in-vitro* conditions to generate tissue to replace a damaged or diseased organ/organ part. With an escalating need for organ transplant, TE has become the need of the hour. Successful growth of cells has been achieved *in-vitro* however, its 3D growth calls for appropriate scaffolds which can form the cellular matrix in the tissue. An ideal scaffold should be biocompatible and display biodegradable properties to match the cellular generation *in-vivo/in-vitro*. Its surface geometry must provide a niche for cellular proliferation, homing, and differentiation, while avoiding elements that develop toxicity to growing cells. The biopolymer unit should be malleable enough to be designed into a 3D structure that facilitate the cell bedding and its regulated

proliferation, enables circulation of media containing a cocktail of nutrients, respiratory gases, growth factors, and also facilitates removal of cellular metabolites. It should also display mechanical properties of the *in-vivo* extracellular matrix of the targeted organ to which it is to be grafted. Several biopolymers of cellulose, starch, collagen, silk, lactic acid have been employed to develop films, solid and hollow fibers, gels, sponges, 2D and 3D constructs suitable for *in-vitro* system based on their biocompatible and biodegradable properties (Eltom et al. 2019).

Fabrication Technique

Numerous cell-manipulations for tissue re-generation, polymer-based biomaterials are being used that can be fabricated by various techniques like Leaching, Freeze drying, Solvent casting/leaching, Robotic deposition, Cross-linking, Electrospinning, and 3D bioprinting.

Electrospinning (ES) is one of the most frequent and adaptable technique for producing fibrous materials, both in the laboratory and on a large scale. ES nanofibrous scaffolds is predominantly intriguing owing to the benefits of nanoscale morphology (like high surface to volume ratio (S/V), biomimetic, capacity of molecular level cellular interaction) along with ES technique's great adaptability, cost-efficiency, and simplicity. ES is generally the preferred process for constructing innovative as well as scaffolds with controlled characteristics that bio-mimic ECM for cell culture. In order to improve the process to overcome the issues like low spinnability potential, difficulty with disrupting the biopolymer's original structure, suitable cross-linking can be done to generate a hydrophobic biopolymer. The ES method enables for fine control of physicochemical properties such as fiber shape and diameter, orientation, volume distribution, as well as end product porosity and thickness. Unfortunately, the most significant disadvantage of the ES approach is that it requires meticulous adjustment of production parameters for each polymer-solvent system in order to appropriately manage the process and the resulting end product qualities. Because the viscosity, electrical conductivity, and other essential characteristics of the polymers and their solutions differ, their behavior during ES also vary, even during the identical circumstances. Unwanted materialistic flaws, for instance, formation of droplets and beads, lack of producing consistently similar product as well as absence of solution spinnability, can be a result of unoptimized process. Figure 3 describes the assembly of an ES instrument along with its working (Haider et al. 2020).

Biologically inspired engineering opens new ground for researchers to develop prototypes to overcome various challenges of research in the field of biomedical engineering and regenerative medicine. Multi-cellular tissue and organs, have highly specialized cells each with its own set of biological tasks that must be replicated in transplanted tissue (Fig. 4) which seems achievable through 3D bio-printing of organs involving different primary cell deposition in a pattern that can closely resemble the natural order of the architecture of desired organ to be transplanted. Post-printing, the cells must retain their *in-vivo* activity under optimum conditions.

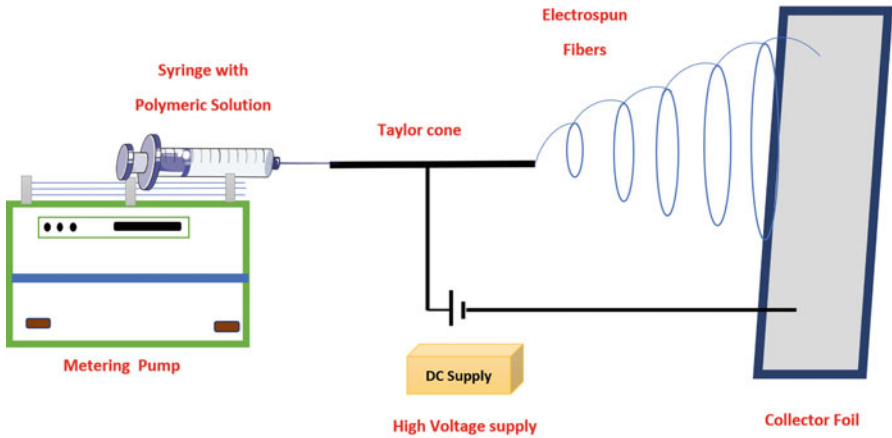


Fig. 3 Electrospinning (ES) Technique- Polymer filled syringe attached to a syringe pump, a high voltage supplied metal needle as well as a collector are the basic components of ES device. On subsequent exposure to electric field, the surface tension of the polymer solution, at the tip overcomes the electrostatic field produced by the field at the revolving mandril resulting in formation of the Taylor cone. Expanded and elongated jet of charged polymeric solution is deposited on a collector in fibrous manner

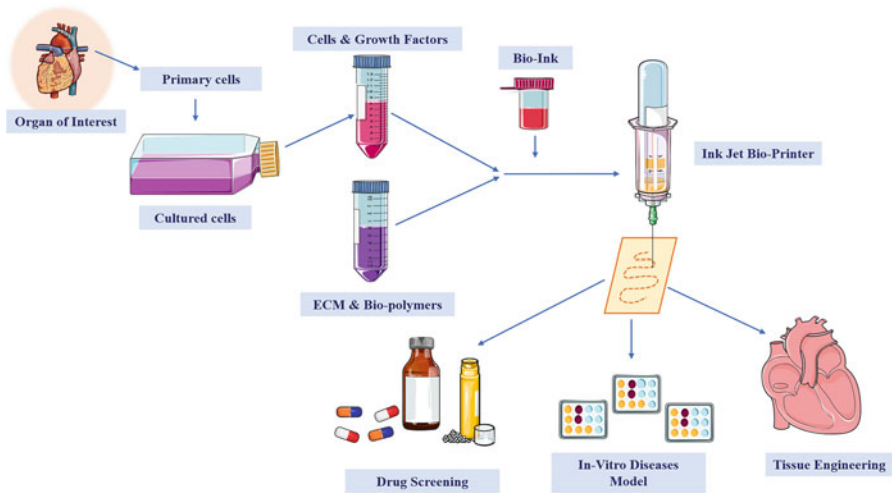


Fig. 4 3D Bioprinting technique- Cells are cultured and subjected to printing in combination with growth factors, ECM, biopolymers, conjugated with Bio-ink

Any cell type used during the purpose of 3D-bioprinting should have a higher extent of cell division and precise cell-growth because lower the cell proliferation higher is the rate of loss of cell viability whereas, uncontrolled proliferation can result in apoptosis or hyperplasia. Furthermore, bio-printed tissues must retain cellular

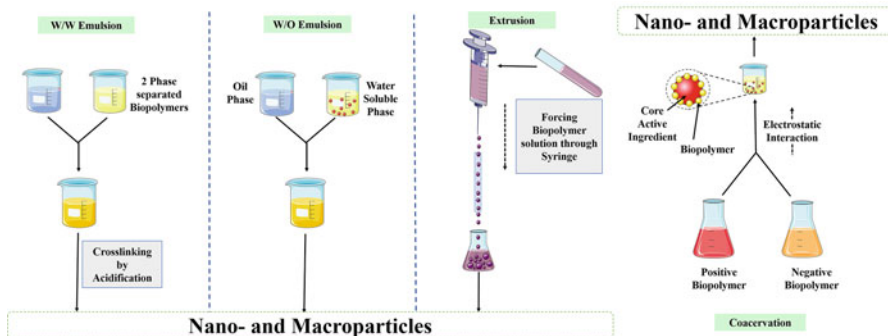


Fig. 5 Fabrication of Nano- and macroparticles (a) Top to Bottom (b) Bottom to Top

homeostasis, self-renewability and react to tissue damage or injury in order to operate long-term after transplantation. Rejection of bio-printed constructs by the host immune system is the major drawback. To overcome this setback, employing an autologous source of cells or through tolerance-induction procedures one can delay graft/ implant rejection.

Nanotechnology has been utilized to fabricate particle-based systems for a wide range of biomedical applications due to its characteristic of encapsulating and releasing active biomedical substances like drugs or therapeutics. Depending on the physiochemical method employed, the fabrication process can be divided into either top-to-down approach or bottom-to-up approach. Milling, shredding, and homogenization are common top-to-down techniques that entail break down of bulk solids or liquids into smaller particles. To break down the particles, these methods typically use three forms of disruptive forces: compression, impact, and shear. Although frequently used in industrial applications to create biopolymer particles, their significant drawbacks include high cost of operation, equipment maintenance, as well as problems in producing particles with well-defined structural features. In contrast, the bottom-to-up methods are characterized as the self-organization of molecules resulting in changes of environmental parameters like concentration, ionic strength, pH, and/or temperature (Joye and McClements 2014). Examples of bottom-to-up method includes coacervation inclusion complexation, liquid ant solvent precipitation and fluid-based gel formation (Fig. 5). Bottom-to-up procedures, on average, have lower energy needs than top-to-down methods. Manufacturing of extremely small particles with better control over particle attributes including size, morphology, and physical state can be achieved by bottom-to-up techniques (Elbert 2011).

Biopolymer and Advancement in Tissue Engineering

In the early 1990s, Langer and Vacanti found “tissue engineering” by application of material science in biology for development of 3D functional scaffolds that can be used to grow cells at the sites of damaged tissue (Langer and Vacanti 1993). This

opened up the new possibilities for regeneration of tissue, with investigators speculating that scaffolds may provide organizational stability and micro-environment conducive for cellular regeneration, emulating the functions of natural tissues. Since then, 3D matrices or scaffolds have been tested for a wide range of applications, including regeneration of tendon/ligament, muscle, neuron, bone, and much more (Eltom et al. 2019).

Specific Properties of Biopolymers Used in Tissue Engineering

Initially natural polymers were employed, owing to their improved overall interactions with numerous cell types without eliciting negative immune response. Synthetic polymers [PLGA, PLLA, and PGA] are frequently combined with natural polymers to enhance the desirable properties such as hydrophilicity, cell adhesion, and biodegradability. Furthermore, specialized ligands, such as protein molecules, are used to boost biological responses on the scaffold surfaces. 3D scaffolds of various consistency like hydrogels, nanofibers, and sintered microparticles have been extensively studied using synthetic and natural biomaterials (Stratton et al. 2016). Post implantation to enhance cellular proliferation and differentiation, these extremely porous 3D scaffolds have proven to be effective. Also the method of fabrication and scaffold geometry may provide the diverse properties-mechanical characteristic, degradation rate besides cellular adherence (Narang and Boddu 2015).

A 3D scaffold can be functionalized in a variety of ways; the majority of which are dependent on the material. A minimum of three functional features are currently regarded to be essential for a 3D scaffold:

- A) Porosity inside a 3D scaffold is a key deciding element for second-generation tissue engineering, which emphasizes the necessity for vascularization and cellular ingression into the scaffold pores. The size and density of the porosity inside a scaffold is one of the determining factors for cellular homing, proliferation and differentiation because cellular networking relies on exchange of nutrients, respiratory gases and metabolites, to ensure adequate cell signaling at various stages of cellular development (Murphy et al. 2013).
- B) Mechanistic property- An ideal scaffold should be tailor-made to maximally mimic the original Extra-Cellular Matrix (ECM) environment, and ensure appropriate surface to volume ratio without compromising its mechanical strength.
- C) Biocompatibility-Scaffold used in TE should be biocompatible and elicit meagre to no immune response. Many different polymeric materials have been surface-modified to add essential molecules for cell growth as per its requirement during various stages of cellular development. To address the constraints of the parent polymer, co-polymerization using the same monomers helps to functionalize the polymeric scaffolds to improve cellular adhesion (Liu et al. 2021).

Applications in Tissue Regeneration

Regeneration of Tendons/Ligaments

Tendons are connective tissue that connects muscle to bone while ligaments connect bones. Tendons do not normally repair effectively after damage, and even mild injuries might end up making the healing process more difficult. Surgical treatments, such as tissue grafts possess inherent complications like graft necrosis, arthrofibrosis, besides failure to restore the mechanical and structural quality of the original tendon. Hence, 3D scaffolds may provide respite to such setback for tendon regeneration to increase the function of de-novo tissue by allowing in-situ restoration (Vasiliadis and Katakalos 2020). Tendon ECM is largely made up of type I collagen and is arranged in a complicated structure that is interwoven, hence making it challenging to recreate 3D biomimetic environment. The Achilles tendon regeneration appears to be a major impediment, attributable due to the persistent mechanical pressure exerted on it (Shearn et al. 2011). Collagen has been used as a scaffold for this regenerative application. Polymeric materials with extraordinarily strong mechanical characteristics, such as silk and PLGA have been used in scaffolds for all tendon regeneration applications (Goh and Sahoo 2010).

Anterior Cruciate Ligament (ACL) plays a strategic role in knee and ligament stability. ACL is mostly formed of collagen types I and III fibers that surrounds the fibroblastic cells. Damage to ACL affects the knee mobility and is now considered a challenging niche in the area of tissue engineering. Polymeric scaffolds like silk fibers and PLLA are being employed for distributing growth factors and imitating the mechanical qualities of the natural environment of tissue since ACL has poor healing capacities and requires outstanding mechanical stability (Laurencin and Freeman 2005). A unique ACL regeneration scaffold has recently been reported, consisting of PLLA nanofibers that are mechanically strong as well as combined with a flexible outer covering of electro-spun PCL nanofibers, allowing for controlled release of growth factors like platelet-derived growth factor (PDGF), fibroblast growth factors (FGF), as well as proliferation of ACL cells (Stratton et al. 2016).

Muscle Regeneration

Muscle tissue regeneration has proven to be a tough task, owing to the fact that scaffolds must maintain integrity of the structure while also being able to generate significant contraction and aid in regeneration.

Muscles are generally classified as smooth, skeletal and cardiac muscle. Smooth muscle tissue is mostly found in the walls of organs, skeletal muscle fibers form unique connections to bones for moment execution while the cardiac muscle tissue is usually forming the heart wall. Cardiac muscle is formed of several cardiomyocytes and has normally very low natural regenerating ability in most mammals. Damage to cardiac muscle cells may result in heart failure. *In-vitro* cardiomyocytes can be nudged to proliferate and differentiate to form cardiac tissue using polymeric scaffolds (Shimizu et al. 2011). Cardiomyocytes are routinely seeded onto alginate, collagen, gelatin, or PGA scaffolds in a 3D structure may lead to higher bioactivity

and cardiomyocyte regeneration. These scaffolds have shown to improve binding to (Arginyl-glycyl-aspartic acid) RGD motifs leading to cellular functioning (Shachar et al. 2011).

Smooth muscle cells develop from quiescent phenotypes to attain adult morphology. This possess barrier for application in engineering tissues, particularly when reverting to a contractile phenotype (Beamish et al. 2010). Nanofibers which are formed of collagen and PCL have been proven to be predominantly beneficial for TE of smooth muscle scaffolds because their orientation guides cell development and allow cells to keep a normal phenotypic shape (Venugopal et al. 2005). Cross-linking, on the other hand, may be used to improve mechanical qualities and promote smooth muscle formation and proliferation required directions.

Skeletal muscle has the ability to substantially regenerate; thus tissue lost during wear and tear is constantly being healed, and replaced without the need for external intervention. However, in the case of a serious damage, its regenerative potential may be reduced or eliminated entirely. Satellite cells play a vital role by migrating to the wound site where it proliferates and differentiates for restoring functional capabilities throughout the repair and regeneration phase (Shearn et al. 2011). Regeneration of muscle is unusual as it encompasses a greater range of tissues. Each tissue comprises of essential components, and there is unlikely to be a single scaffold that can be used to regenerate all tissue types. Myogenic progenitor cells show increased proliferation when subjected to electrical stimuli on a 3D scaffold due to 65% increase in NO(x) which activates satellite cell, suggesting the usefulness of this technique in future (Anderson et al. 2015).

Nerve Regeneration

The Central Nervous System (CNS) and the Peripheral Nervous System (PNS) are the two major parts of the Nervous System (NS). The CNS comprises of the brain and spinal cord, whereas the PNS is formed of the ganglia and nerve tissue located outside the CNS. Both systems have typically been addressed in TE efforts for nerve regeneration. Schwann cells functionally contribute to myelination of axon to provide structural support to the peripheral nerves. These are often targets of grave injury due to diseases or accidents hence, research activities pertaining to Schwann cell regeneration are often at the forefront. For a successful nerve regeneration, the regenerating axons mandate directional cues which are often lost during injury. Hence, bioactive 3D scaffolds are employed to provide axonal guidance and support besides promoting axonal myelination. Nanofibers have specifically aided in nerve guidance conduits, which directs the outgrowth of neuronal axons during the regenerative processes. They are known to have a variety of functions, including their ability to regulate the direction of axonal development, release growth factors that help in tissue regeneration and reduce inhibitory scar tissue. Hydrogels employed in NS regeneration, is reported to promote axonal regrowth via Schwann cell (Y. Y. Tseng and Liu 2015); however the rigidity of the hydrogel scaffold is crucial to its success. Softer hydrogels allow differentiation of neural stem cells into neurons and astrocytes whereas stiffer hydrogels helps them to differentiate into oligodendrocytes (Shoichet 2010).

Regeneration of Skeletal Tissue

Bone grafts, which have historically been utilized for repairing osteogenic deformities, are being phased out in favor of synthetic polymeric scaffolds for TE purposes. Because of their potential to produce reactions at the cellular level, nanostructured materials have become popular for bone regeneration. Due to structural similarities, beta tricalcium phosphate, bioactive glass and hydroxyapatite are commonly employed scaffolds in bone regeneration (Damien and Parsons 1991). The benefits of such inorganic materials appears to have higher potential for osteoconductivity and compressive strength (Schmitz et al. 1999). Mechanical strength that replicates native tissue is a significant element in bone healing. As a result, high-mechanical-strength polymeric materials, such as chitosan nanofibers, PLLA, bioactive glass material and silk protein are frequently employed as scaffolds in bone regeneration. Due to high compressive strength, fatigue resistance and porosity, metallic scaffolds, such as those made of titanium, have become quite popular (Dabrowski et al. 2010). Although this scaffold has been successfully applied in osteogenic regeneration; However, vascularization and mechanical strength remain enigmas in the area of bone regeneration, despite ongoing research to improve these characteristics.

Inadequate vascularization has been shown to result in a severe lack of necessary nutrients for cell survival inside a scaffold, as well as unanticipated and harmful abnormalities in differentiation has been reported. Injecting particular growth agents like VEGF (Wernike et al. 2010) into a scaffold is one way to encourage vascularization. PLGA scaffolds have been recognized to be acceptable options for bone TE owing to their mechanical strength and ability to provide an environment favorable for vascularization, when combined with other bioactive materials for increased cellular adhesion (Ishaug et al. 1997).

Biopolymer Application in Organ Culture

The biomedical cost of treating chronic injuries continue to rise in lockstep with population growth. Clinical results in this area remain unsatisfactory despite cutting-edge medical and surgical treatments because the availability of total number of organ donors do not meet the required need for replacement of biological parts.

In case of burn victims, the decisive goal is to develop materials that endorse efficient skin regeneration simultaneously checking microbial invasion and providing aesthetically acceptable characteristics especially with scar-free results (Venugopal et al. 2005). As a simple, adaptable, and effective approach, electrospun nanofibers have garnered a lot of interest as a possible scaffold for wound dressing as well as bio engineering (Mogoşanu and Grumezescu 2014). Due to their inherent properties, such as high S/V ratio, porosity, permeability tunable mechanical properties, and resembling ECM promoting cellular migration, adhesion, and proliferation, ES nanofibrous scaffolds have many advantages over other types of scaffolds (Venugopal et al. 2005). Skin restoration has been studied using synthetic and natural fibrous membranes made of alginate, collagen, chitosan, cellulose, gelatin,

PVA, PLA, and PVP. Despite having high mechanical qualities, they are usually hydrophobic and have poor cellular adhesive properties, which make them unsuitable for cellular development (Mahmoudi et al. 2017). In contrast, natural polymers are found to have superior biocompatibility and biodegradability than their synthetic counterparts, although their mechanical qualities deteriorate rapidly during decomposition. As a result, the hybrid fibrous scaffolds that combine the benefits of natural polymers with the mechanical robustness of synthetic polymers have made substantial progress in skin TE. Chitosan, possesses biodegradability, biocompatibility, and inherent antibacterial activity making it an ideal candidate for skin grafts as well as its chemical structural resemblance to glycosaminoglycan in the ECM and hemostatic action, make it a suitable for skin replacements (Jafari et al. 2011).

Cornea is the transparent outermost layer protecting eyes from infection and also provides mechanical strength. The transparent stroma which accounts to 90% of the thickness of the corneal layer is essential for vision. Substantial damage to the stromal layer may lead to corneal blindness. This is either treated with a donor cornea transplant or requires an implantation of artificial cornea (Matthyssen et al. 2018). During ocular damage, corneal regeneration mediated by stem cell and TE are now a promising tool to overcome such problems. Numerous research investigations examining the development of tissue-engineered epithelial, stromal, and endothelial replacements have been conducted due to a scarcity of donors and the keratoprotheses' limited application potential (Hollick et al. 2006). The most significant characteristics for corneal scaffolds are biocompatibility, transparency, and strength. Furthermore, scaffold-based techniques focus on the fabrication of a replica that can mimic original tissue architecture and microenvironment that enables cell adhesion, proliferation, differentiation and migration (Kilic Bektas and Hasirci 2018). Due to malleable mechanical properties synthetic polymers stand out, to be suitable for designing corneal stroma. Also these scaffolds have the ability to induce keratinocyte development in human stromal cells. In order to differentiate stromal stem cells to the keratinocyte lineage, polyester urethane has been employed as a scaffold in conjunction with stromal stem cells (Ghezzi et al. 2017). Collagen type I, Chitosan, PLA and PEG have superior biological, mechanical and optical properties in contrast to available synthetic polymers. In order to achieve better optical properties a blend of natural and synthetic polymer is widely employed (Ahearne et al. 2010). Type I collagen, a biopolymer component of stroma, offers advantages of cell encapsulation and corneal stroma regeneration when used as corneal scaffold. However, the major setback is poor mechanical strength. Chemical cross-linkers might help to mitigate the mechanical flaws in collagen type I hydrogels (Kubrak-Kisza et al. 2016).

Nanocellulose is a unique biodegradable polymer that has the potential to revolutionize research and development in a variety of biomedical fields. It encompasses characteristic like toughness, ability to be fabricated into thin films or elastic gels making it an ideal biopolymer for producing wound dressings. Adam and team from Swansea University, Swansea, UK, formulated grid wound dressings with TEMPO (2,2,6,6-tetramethylpiperidine-N-oxyl) and C-Periodate nanocellulose bio-inks, with the deposited components forming a porous structure. They concluded C-Periodate

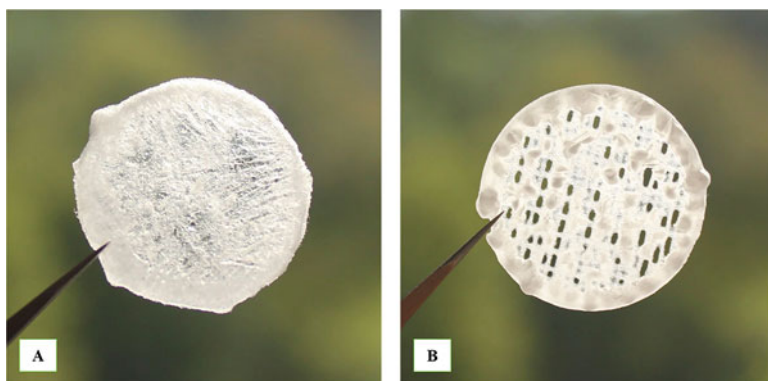


Fig. 6 (a) TEMPO Nanocellulose. (b) C-Periodate nanocellulose. (Image reprinted with permission -Copyright © 2015 Adam Rees et al. 2015)

nanocellulose which is suitable for use as bio-ink based for the printing of grid structures mostly owing to its consistency and rheology. The nanocellulose produce porous 3D structure having the ability to carry drugs and also checks antibacterial growth which significantly improves wound dressing (Fig. 6) (Rees et al. 2015).

Lab-on-Chip

Biomedical Lab-on-Chip (LOC), is a micro-fabricated device based on Micro-electromechanical Systems, which use a dynamic 3D environment comparable to that of the human body. LOC presents a multitude of opportunity to better understand the etiology of human illnesses and provide a potentially superior model for drug screening. Although organ-on chip models have their limitations, the introduction of new technologies has improved their capabilities for translational research and precision medicine. Microfluidics is the study involving fluidic manipulation at micro/nanoscales as well as the engineering of microfluidic devices, design helping in study of the fluidic model, fabrication of transport and delivery mechanism as fluid movement remains the foundation of Biological or Biomedical Micro-electromechanical Systems and the concept of Lab-on-Chip. Here the biochemical reactions are comparatively faster at lower volume due to lesser but faster utilization of enzyme-substrate and also reduces the experimental cost. LOC gives several advantages like faster diagnosis, cost effectiveness, reduced amount of sample usage, and patient-specific on real time basis. In the revolution of LOC various organ on chips (Fig. 7) have been developed like lung (Artzy-Schnirman et al. 2019), brain (Koo et al. 2018), liver, kidney (Ashammakhi et al. 2018), heart (Ferrari and Rasponi 2021) which it can bio-mimic the functions of organ as well as can serve as a model for diseased condition.

Kimberly and co-workers from Harvard University, (MA), USA, developed a 3D convoluted renal proximal tubule (PT) on a chip. Perfusable PT was designed using

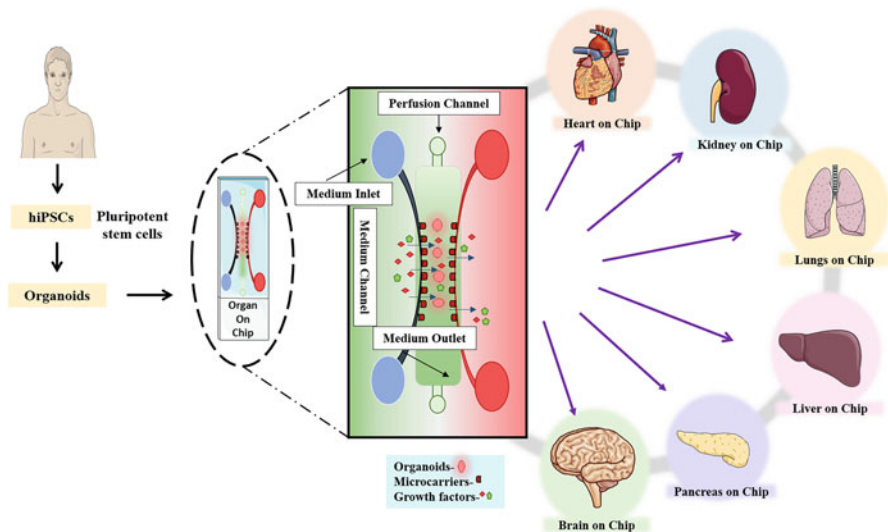


Fig. 7 Organ on chip- This schematic diagram shows the development of organ on chip using human pluripotent cell aggregates (organoids) flowing through microfluidic channel of medium contain growth factors and ECM

fugitive ink which is first printed on to gelatin-fibrinogen ECM followed by ECM casting around the printed bio-structure. Fugitive ink is then retracted to create a hollow tubular structure that resembles normal renal tubule. PTEC-renal proximal tubular epithelial cells are then infused in the tubule and perfused for an extended time period. This 3D PT models was investigated to treat drug induced tubule damage (Homan et al. 2016) (Figure 8).

Microcarrier Beads

For 3D cell culture- solid, porous biodegradable polymer-based scaffolds are developed and tested for cellular adhesion and development. Microcarrier beads comparatively provide greater biocompatibility and porosity imposing efficient 3D cell cultures as it provides efficient cellular adhesion with higher viability, cellular proliferation and ECM remodeling. Technological challenges are imparted in achieving normal cellular cytoarchitecture as well as tissue construct. With a greater surface area to mass ratio, microcarrier beads are preferred as a go to material for cellular adhesion in-consideration with cell density and cellular yields compared to scaffold at macroscopic scale. Porous beads provide a better gaseous and nutrient exchange, also metabolic wastes may be transported more easily within the microcarriers than in cellular spheroids. Due to their benefits such as high specific surface area, suspension culture with homogenous stirring and simple scale-up, it has demonstrated promising ability to grow a wider range of mammalian cells in high yield.

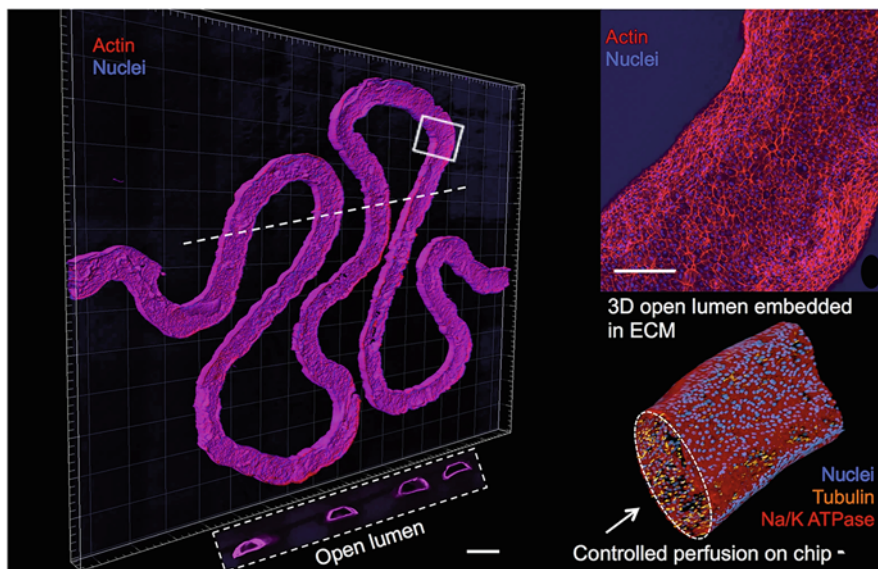


Fig. 8 3D convoluted renal PT on a chip. Reproduced with permission from Homan KA, Kolesky DB, Skylar-Scott MA, et al. Bioprinting of 3D convoluted renal proximal tubules on perfusable chips. *Sci Rep.* 2016;6:34845. Copyright © Nature Publishing Group. <http://creativecommons.org/licenses/by/4.0/>

Biopolymers and Its Role in Targeted Drug Delivery

Drug Delivery Systems (DDS) are methods for delivering drug candidates to specified region of the body for controlled release. Here the drug particles include beads, bio-composites, dendrimers, films, gels, hydrogels, implants, microparticles, nanoparticles, microcapsules, micelles, nanocapsules, patches, and spheroids scaffolds. Biopolymer carrier intended to carry therapeutics to the targeted site plays an important role in the preservation of drug. Damage and/or degradation of proteins and peptide molecules can affect their chemical structure (Jacob et al. 2018) resulting in the inactivation of drug molecule or may even prevent them from reaching their target sites. A model drug delivery system should ideally be biocompatible, possess high drug carrying capability, should be non-toxic and preferably have simple usage mechanism (Saqib Hasnain and Nayak 2018). While creating a DDS the primary channel lies in selection of polymers which may satisfy the aforementioned properties. Enzymatic or nonenzymatic biodegradation of biodegradable polymers occurs *in-vivo*, yielding biocompatible or nontoxic by-products (Doppalapudi et al. 2016). This polymer ideally enhances medication pharmacokinetics while lowering adverse effects. Recent advances in controlled drug delivery systems (CDDS), sustained release drug delivery systems (SRDDS), anticancer drug delivery, nucleic acid, protein, vaccines as well as TE and regenerative medicine, have focused on

biodegradable biomaterials (Glieder et al. 2015). In the concept of novel macromolecules in targeted drug delivery application, biodegradable polymers have a notable focus on its chemistry. Today various polymers of natural as well as synthetic classes have been utilized for various DDSs.

Hyaluronic Acid

Hyaluronic acid (HA) is a biodegradable natural biopolymer which is highly biocompatible. HA has garnered a lot of interest as a tool for developing DDSs because of its mucoadhesive properties and non-toxic nature. HA and its derivatives are well suited for targeted drug release due to its elasticity along with viscosity, ability to bind to surface receptors of the targeted cells of various organs including kidney, liver as well as tumor cells. It has been employed in administration of various non-invasive and invasive medication administrations. The DDS via nasal as well as ocular routes are examples of non-invasive applications. On the other hand, its usage in invasive treatment methodology includes subcutaneous injection of protein medicines with a sustained drug release (Huang et al. 2020).

Hyaluronic acid (HA) scaffolds are employed in regeneration of both hard and soft tissue. HA hydrogel has been most commonly utilized in TE owing to its capacity to encapsulate cells and other components for distribution purposes, as well as its swelling characteristics. Besides hydrogels, HA scaffolds can be manufactured by electrospinning to build a fibrous scaffold (Séon-Lutz et al. 2019) or by co-polymerizing other biomaterials to manufacture scaffolds that are porous by leaching salt particles (Liu et al. 2021), Rapid Prototyping, Thermal-induced Phase Separation, 3D Printing Phase Separation, Stereolithography, Selective Laser Sintering, Solvent-based extrusion Freeforming and Fused Deposition Modelling (Eltom et al. 2019). Wet spinning, a unique layer-by-layer spray-assisted kind of assembly process is employed to layer different films of polyelectrolyte onto porous HA scaffolds during *in-vitro* culture of human keratinocytes (Montero et al. 2021). Finally, 3D printing seems to be a proven technology for producing HA scaffolds, since it allows microscale reproducibility of scaffold properties, potentially leading to enhanced cellular function as observed in animal models (Gaetani et al. 2015).

Gelatin

It is abundantly present as Type A or B in almost all the animal tissue. Being a natural polymer, gelatin possesses high bio-compatibility as well as low antigenicity. Using a burst or controlled release mechanism, gelatin NPs can offer controlled medication delivery. High solubility as well as poor mechanical strength limits their usage in the field of DDS. To overcome its shortcomings, gelatin can be functionalized by copolymerizing with chitosan composites or by addition of cross-linkers, such as alginates (Sharma et al. 2015) or incorporating it into a synthetic polymer like PVA (Afsana et al. 2019) that enhances its properties as an ideal polymer for DDSs.

Chitosan

3D chitosan scaffolds exhibit functional and structural features identical to glycosaminoglycans, a natural lubricant found in the human body. Previous studies have attested to its bioactivity which stimulate cellular adherence naturally (Candy and Sharma 1990). Therefore, 3D scaffolds of natural polymers combined with chitosan or alone, are utilized in gel, fiber or sponge forms due to its multifunctional structure and capacity to cross-link in TE applications (Azizian et al. 2018). Chitosan has a low solubility at physiological pH, which allows it to be used for elongated period (Albanna et al. 2013).

Polycaprolactone

Polycaprolactone (PCL) first synthesized in 1930s, is a synthetic polymer frequently employed for the fabrication of suitable scaffolds for TE. The PCL structure is formed of five non-polar methylene groups besides one semi-polar ester group. PCL is found in a variety of forms including microparticles, fibers, and films but in order to improve its bioactive properties for various tissue regeneration applications, it is used in the form of composites with co-polymers, like chitosan and gelatin (Arun et al. 2021). In contrast, with other available polyesters, PCL has higher drug permeability, moderate rate of degradation, and fewer acidic by-products, thus making it advantageous in the domains of drug administration and TE. Also, PCL has shown to have higher solubility and blending ability with other biomaterials (Wright et al. 2010). However, PCL possess weak cellular adhesion capabilities, the limitations showcased due to its lower disintegration rate making it an unsuitable scaffold for short term application in TE. To counteract this disadvantage, a variety of techniques have been tried, including- copolymerization and surface functionalization. PCL was initially investigated as a vehicle for constrained medication and vaccine administration due of its low toxicity and high drug permeability. PCL thin film devices are inserted subcutaneously for local or systemic distribution due to its flexibility, biodegradability, and biocompatibility (Narayanan et al. 2016).

Poly-Glycolic Acid

Poly-Glycolic Acid (PGA) was initially found as a biomedical degradable polymer. It has the capacity to increase the efficacy of drug formulation for DDSs while providing a regulated release pattern. PGA is employed in DDS owing to its strength, physiological capability, mechanical properties and non-toxicity. Under physiological settings, polyglycolide degrades due to the non-specific breakdown of esters. Within the body, polyglycolides get either eliminated in urine or gets converted to water and CO₂. However, PGA is found to degrade quickly and remain insoluble in several common solvents, severely inhibiting its application. Yet it offers an arena for discovery of better methods than current protocols (Singh and Tiwari 2010).

Polyurethanes

Polyurethanes (PUs) are flexible polymers with carbamate or urethane($-\text{NH}-\text{COO}-$) linkages in their chains that are biocompatible and physiologically stable. Biodegradable polycaprolactone segments in biodegradable PUs are employed in tissue repair implants and medication delivery systems. To get a favorable drug release profile, physicochemical characteristics of PUs may be adjusted by varying the amount of water sensitivity and crystallinity. The addition of an ionic group promotes electrostatic interface of charged drug molecules with DNA/RNA or proteins. PUs has been investigated for use in biomedical research, however its stability restricts their utility (Cherng et al. 2013).

Polyethylene Glycol

The Poly-Ethylene Glycol (PEG) is the most widely used non-ionic hydrophilic polymer that displays stealth properties which shows reduced agglomeration and enhance stability during storage and application. PEG is used for drug delivery and probably the only polymer having multiple drug approvals. PEG's hydrophilicity, low interaction with blood components, and high biocompatibility all contributes to its success. Yet research findings also warn us about its downsides that includes undesired interaction with the immune system, probable breakdown under stress, and systemic retention beside an unpredicted excretion limit (Knop et al. 2010).

Poly (lactic acid-co-glycolic acid)

The Poly (lactic acid-co-glycolic acid) (PLGA), extensively utilized biodegradable synthetic polymers for TE application is an amalgamation product of PLLA and PGA. The greater the ratio of PGA in a PLGA scaffold, lower is the half-life of PLGA. Benefits of PLGA includes biodegradability, flexibility and fabrication of various types of formulations, and surface modification for DDSs (Gentile et al. 2014). Unfortunately, PLGA also has a significant flaw that limits its bioactivity. It's breakdown firms highly acidic by-products i.e. glycolic acid and lactic acid which can be difficult for the human body to quickly digest in large concentrations. Therefore, in the presence of acid-sensitive pharmaceuticals, this can pose to be a problem in drug delivery applications. Altering the PGA:PLLA ratio such that there is more PGA present, results in a slower degradation rate and has less acidic by-products. However, it is reported that in the presence of certain salts the pH of PLGA by-products may rise, resulting in higher overall bioactivity (Gentile et al. 2014). PLGA has been tagged as "smart polymer" due to its stimuli sensitive properties. PLGA has been utilized for sustained drug release, drug delivery, imaging (Rancan et al. 2009), and protein delivery. It has been extensively used for encapsulating anticancer medicines, quantum dots, and production of magnetic nanoparticles.

Cellulose Acetate

Plant cell wall contains cellulose from which cellulose acetate (CA) is derived. CA is a non-toxic biomaterial that is biocompatible and biodegradable. It has a cheaper production cost, is less hygroscopic and has good chemical and heat resistance. The cellulose derivatives- cellulose triacetate and cellulose diacetate, readily dissolves in organic solvents and can be potentially used in a variety of industrial and biological applications. TE (Rubenstein et al. 2010), antibacterial applications, DDSs and wound dressings (Samadian et al. 2018) are a few of the biomedical application of electrospun CA nanofibers. The use of CA in DDSs is linked to a variety of their features like good electrical conductivity and rapid expulsion from syringe needle under high electrostatic stress and random deposition as nanofibers on a collector. Antioxidants, nonsteroidal anti-inflammatory medications (NSAIDs), and vitamins could all be delivered effectively using electrospun CA nanofibers (Taepaiboon et al. 2006).

Polyanhydrides

Polyanhydrides are a kind of surface eroding hydrophobic polymer that has two carbonyl groups connected by an ether bond. One key benefit of bio-erodible controlled release systems over most other types of delivery systems is that the drug release is potentially regulated mostly by the polymer's characteristics features than the medication itself. This makes biodegradable polymers capable of releasing even macromolecules like proteins. As most of the water molecule cannot permeate till the polymer erodes, the drugs can be preserved when loaded with such biodegradable polymers. Such technology would have a huge influence on the efficacy of protein-based medications. The creation of formulation processes that would keep the protein in its active state during device construction nevertheless remains a major problem. Antineoplastic agents (Eameema et al. 2014), local anesthetics, anticoagulants, neuroactive medicines, antibiotics, vaccinations (Ghadi et al. 2017), and proteins (Sharma et al. 2015) have all been studied for delivery of therapeutics.

Polyethylene Vinyl Acetate

Polyethylene vinyl acetate (PEVA) is a transparent thermoplastic derived from combination of polymer of ethylene and vinyl acetate monomers. PEVA is a well-studied and commercially available excipient for long-acting dosage formulations. It is a preferred and well-studied commercially available biopolymer with proven track records for the formulation of complicated, long-acting dosages and which can be tailor made to deliver different therapeutics using various production methods. A wide range of pharmacological agents, biological molecules including peptides, nucleic acids and proteins, including monoclonal antibodies and growth factors

can be delivered using PEVA-based DDS (Major et al. 2019). PEVA has been reported to be an inert molecule and hence found to be relatively stable in the *in-vivo* system (Externbrink et al. 2013).

Polysiloxanes

Polysiloxanes is a unique polymer which is known to exist in branched, cyclic ring, linear and cross-linked in 3D network. Its unique physicochemical properties rely on its organic nonpolar functional groups and polar inorganic backbone. Silicone fluids of volatile nature which consist of straight linear chain of active or excipient pharmaceuticals on the other hand nonvolatile silicone fluids have shown application in the cosmetic industry (Blanco 2018).

Poly Di-Methyl Siloxane (PDMS) is a good example of this class of polymers. It shows a wide range of applications due to its inertness, biocompatibility, meager toxicity, thermal and oxidative stability, low modulus and antiadhesive properties and good organic solubility. It also displays better potential to form films, adequate adhesion to various substrates, and excellent resistance to chemical degradation both as an active substance (artificial skin, blood pumps, cardiac pacemaker leads, contact lenses, DDSs, denture liners, medical adhesives, and maxillofacial reconstruction) and as an excipient (adhesive pressure-sensitive and skin patches) in medical devices (Abbasi et al. 2001).

N-(2-hydroxypropyl)meth-acrylamide (HPMA) Copolymers

HPMA are non-biodegradable polymers with high biocompatibility. Several functional groups may be added into the polymer backbone such as drug moieties resulting in biologically active HPMA copolymers. Addition of antibody or antibody fragments lowers the immunogenicity of the biopolymer making it more biocompatible. Currently HPMA is one of the most explored platforms for polymer–drug conjugates and its primary application has been heralded to be carrier of anticancer medicines, site-specific DDS to the GI tract antisense oligonucleotides specific to tumor (Jensen et al. 2003).

Application of Biopolymers in Hemostatic Gauge and Dressings

Skin is majorly composed of epidermal layer containing keratinocytes followed by the dermal layer predominantly comprised of high collagen containing dense connective tissue. Skin damage leads to wound formation which triggers a series of complex events to attain the skin integrity as shown in Figure 10. Wound healing (WH) is well-known to contain a series of four overlapping phases via Hemostasis,

Inflammatory, Proliferative and Remodeling phase. The **hemostasis phase** predominantly involves platelet aggregation and series of event required for plugging the damage in blood capillaries to block blood loss from the wound site. This ensures sufficient period for immune cell recruitment and signaling molecules for the commencement of **inflammatory phase** (Chen et al. 2018) much needed to remove tissue debris and invading bacteria by the process of phagocytosis initiated by infiltration of neutrophils as well as monocyte. This is followed by the **proliferative phase** wherein the wound tissue area is diminished by contraction and fibroplasia to result in wound closure. This phase involves important events like angiogenesis, vasculogenesis, fibroplasia, and re-epithelialization. This phase may last from 48 h to 14 days after the wound formation. The final **remodeling phase** successfully generates ECM which helps in restoring the normal skin morphology; this marks the end of remodeling phase anywhere near 14–21 days of WH post wound formation (Gonzalez et al. 2016) (Fig. 9).

Biopolymers are now employed extensively in WH applications. Their multi-functional behavior and physical state tunability allows them to be used in a broader range of wounds; from woven fibers that have an ability to expand when they come in contact with the exudates to water-rich hydrogels that maintains moist environment to avoid wound drying. There is a boundless array of physical characteristics that can be tailored for a given usefulness because of the huge range of biopolymers available and its ability to blend with other biopolymers. Smart biopolymers that are physiologically sensitive can be produced because of their physical compatibility and capacity to load and release drug controllably when exposed to varied biological environments.

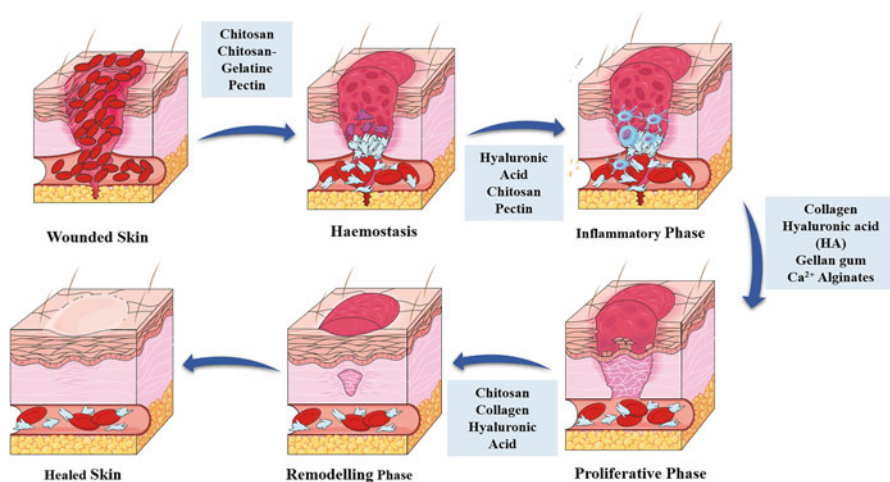


Fig. 9 Stages of Wound Healing – During Hemostasis the platelet aggregation checks the blood flow followed by inflammatory phase which is characterized by neutrophil and macrophage filtration at the site of wound. Proliferative phase is marked by cell proliferation, angiogenesis and neurogenesis. Followed by proliferation is the remodeling phase which helps to restore the natural framework of the wounded skin tissue

Biopolymer as Hemostatic and Anti-Inflammatory Agents

The hemostasis has traditionally been the major focus during wound management. Primeval people used herbs, slimy material and most often sand as hemostatic agents to stop bleeding. Advancement in the sector of biotechnology has introduced applications of various natural and synthetic polymer based hemostatic agents.

Alginate

Alginate based wound dressing has known its way into the biomedical science as wound healing material since 1980s. In its native form, alginate is a heteropolysaccharide made of uronic acid residues of 1 \rightarrow 4-linked β -D-mannuronic (1 \rightarrow 4 M) and 1 \rightarrow 4-linked α -L-guluronic acid (1 \rightarrow 4GA). But during biosynthesis, the polymer chains consist of only residues of 1 \rightarrow 4 M which are converted 1 \rightarrow 4GA; this epimerization is catalyzed by the enzyme C5 epimerase. Epimerization results in a conformational change which in turn enhances physical properties of the polymer. Uronic acid present in alginate-based biopolymer dressing has the ability to bind to metal ions like Na^+ and Ca^{2+} . Calcium salt-based alginates are in-soluble in water and hence can be interlaced into fibers and used as dressing patches or cavity fillers.

Wound exudation is the major problem that needs to be accounted while designing a biopolymer-based dressing. The ability of alginates to absorb 15–20-fold higher volumes of fluids compared to its weight makes it well suited as dressing material (Jones 1999). The proportion of guluronate groups and mannuronate groups tend to determine the swelling. Calcium when comes in contact with wounded area, there is an exchange between Na^+ ions from the exudated wound and Ca^{+2} rich alginate, resulting in solubilization. Since Ca^+ can readily exchange only when loosely bound to mannuronate guluronate, hence higher the guluronate group lower is the swelling (Yimin Qin 2008).

Chitosan

Chitin is a highly available natural biopolymer formed of poly-N-acetyl-d-glucosamine present in exoskeleton of crustaceans and in fungi and bacterial cell wall. Deacetylation of chitin forms chitosan, which is known to possess improved solubility, bioavailability, biocompatibility, and biodegradability. The N-acetyl group is either fully or partially replaced by NH_2 , and these acetylated or deacetylated groups can be distributed randomly or block-wise in chitosan (Vårum et al. 1991). The physicochemical and biological properties of the biopolymer is determined by the composition and distribution of these two units. Previously, chitin-based polymer dressings are well-thought-out to be effective for the hemostasis. Chitosan is currently preferred for developing dressings to control post-surgical bleeding in patients. Chitosan contains positively charged amino group which can readily interact with negatively charged membrane of RBCs (red blood cells) and hence serves as a good anticoagulative agent. Its higher porosity aids in-sufficient gaseous exchange essential for WH. It is argued that chitosan in solid state and solution state have different hemostatic activity (Yang et al. 2008) due to the varying degree of

acetylation (DA). The rate of degradation of chitosan is directly proportional to the DA and inversely proportional to its molecular weight and degree of crystallinity. To overcome this, increased degree of deacetylation (DD) has proven to be an effective strategy. However, it is noted that increased DD causes decrease in its degradation (Islam et al. 2019). Amalgamation of carrageenan in chitosan by carboxymethylation and cross-linking modifications have resulted in dressing with increased hemostatic competence (Pippi et al. 2017).

Chitosan can be processed into many forms like membranes /sponges, hydrogels, beads, films, microgels/ nanogels, nanofibers, micro/nanoparticles or scaffold /bandages. Both biological and mechanical properties of chitosan make it suitable for use as biomaterial. Chitosan based 3D hydrogels are used in tissue regeneration and targeted DDSs due to its soft, flexible and non-toxic properties. However its instability results in dissolution beyond control and despite shows low mechanical resistance. Chemically cross-linked chitosan-based 3D hydrogels have more stability and smaller pore size. Chitosan-based sponges are free standing highly porous and soft material but may shrivel. It is most commonly used as filling material in treating wounds. Chitosan based 2D films used as coating materials but has high production charges and is labor intense. It finds its way into biomedicine as scaffold coating material or wound dressing substitute for skin. 2D porous nanofibred membrane which mimics skin ECM. They are highly porous but during production, electrospinning of chitosan is the major hurdle. Such membranes are used for wound dressing and scaffold coating material (Croisier and Jérôme 2013).

Pectin

Dicotyledon cell-wall constitutes of a large proportion of pectins, heteropolysaccharides which plays an important role in cellular growth and development (Willats et al. 2006). Pectin polysaccharides are structurally composed of regions of rhamnogalacturonan type I (RG-I) and homogalacturonan (HG). Pectin has several recomences as wound dressing materials mainly due to hydrophilicity, which aids in the wound exudate removal (Munarin et al. 2012). Traditionally, pectin-based materials seemed to have been used in wound healing since they maintain an acidic pH that acts as antimicrobial barrier. Pectin potentially binds to bioactive molecules and prevents degradation (Jáuregui et al. 2009). The amount of pectin in the dressing varies depending on the phase of WH $\sim 40\%$ in hemostasis phase and during the inflammatory phase less than 10% pectin is suitable (Alvarez and Lechuga 2010).

Gelatin

Gelatin is abundantly present in mammalian tissue and is generally produced from collagen type I present in skin and bone. Gelatin depending on its isoelectric point are characterized into type A(~ 8.0) and gelatin type B(~ 4.9) (Poppe 1992) and hence contributes to different total charge of the gelatin, at physiological pH. Hence selecting the gelatin material based on the exudate pH is important when used in WH dressing material. High-molecular-weight gelatins which are produced first during extraction of gelatins have higher viscosity along with a greater gel strength compared to lower molecular weight gelatins which are produced later in the extraction process. The

strength of gelatin-based hydrogels is measured in terms of “bloom value,” example commercially gelatins range from 50 to 300 Bloom value. “Bloom value” is directly proportional to strength of the hydrogels. Gelatins serves as wound dressing for a various type of wound starting from partial to chronic wounds. Due to its hemostatic properties and its ability to get easily incorporated with other biopolymers it acts as a promising candidate for wound dressing material. Gelatin based hydrocolloid dressings that containing pectin and carboxymethylcellulose; sterile compressed gelatin-based sponge absorbs 45-folds more blood compared to its weight (Council on Pharmacy and Chemistry, 1947). The limitation to using mammalian origin gelatin despite having attractive wound healing properties is due to ethical factors and religious obligations. Hence, producing gelatin-free hydrocolloid dressings is the only suitable alternative. Targeted growth factor, antimicrobial drug delivery to wound using gelatin is widely studied (Kawai et al. 2000; Rattananuengsrikul et al. 2009; Tanaka et al. 2005).

Hyaluronan

Hyaluronic acid is an important constituent of ECM and found in animal tissues. It is also a constituent of connective tissues like vitreous humor, synovial fluid, as well as dermis and epidermis of skin. It hence plays an important role in various phases of WH.

During the WH, in inflammatory phase, high-molecular-weight HA is seen to be functional and has antiangiogenic and immunosuppressive activity (Delmage et al. 1986), and it also functions to aid granulocytes infiltration for checking bacterial growth and debris of damaged tissues. High-molecular-weight HA molecules occupies a large hydrodynamic volume physiologically also it randomly stiffens when hydrated. This significantly aids to trapping large volumes of aqueous fluid, making it an ideal hydrogel (Gandhi and Mancera 2008). Chitosan based dressings due to the degree of acetylation and molar mass of chitosan impact biological features such as antibacterial, analgesic and enhances permeation, albeit the mechanism of these influences is not apparent and frequently conflicting (Holme et al. 2008).

Agents for Regulation of Angiogenesis, Tissue Regeneration, and Remodeling

Hyaluronic Acid

In biomedical sciences Hyaluronic Acid (HA) was initially used for treatment of corneal wounds, viewing the positive outcomes it was then that commercialization for repairing corneal damage. HAs' have been also used for managing chronic diabetic, leg, and pressure ulcers. HA has emerged as a promising scaffold for skin tissue engineering. Addition of HA benzyl ester in the co-cultured human fibroblasts and keratinocytes shows expression of normal skin molecules in both dermis and epidermis (Gandhi and Mancera 2008). In contrast, with higher-molecular-weight HA the low-molecular-weight HA dominates in remodeling as it stimulates fibroblast proliferation and collagen synthesis (Rooney et al. 1995).

Gellan Gum

It is not seemed to be widely used in WH dressings. Gellan is bacterial origin biopolymer, produced as a product of aerobic fermentation by the bacterium *Sphingomonas paucimobilis* (Gibson and Sanderson 1997). Endotoxin free gellan is a promising material for bio-engineering (Smith et al. 2007). Gellan is composed of a linear tetra saccharide repeating unit of glucose, glucuronic acid and rhamnose. High acyl gellan, the native form, glyceryl and acetyl substituents are linked to the 1 \rightarrow 3-linked glucose residue. In contrast, the low acyl gellan, produced by removing glyceryl and acetyl substituents, can be used for application in WH due to its physical properties. On dissolution in water at around 80 °C gellan forms randomly coiled structure which transit to double-helix structure during temperature drop. This coil-helix transition in conformation is concentration dependent (Miyoshi and Nishinari 1999). In this well-organized state its viscosity increases in to exhibit “weak gel” properties. Gellan with divalent cations like Ca^{+2} forms hydrogels due formation of linkage between double helical pairs (Morris et al. 2010). In contrast, gellan also forms hydrogels with monovalent metal ions such as Na^+ and K^+ . In order to form a cross-linking similar to wound fluid physiology concentrations, of 5 mM Ca^{2+} and Mg^{2+} and 100 mM Na^+ and K^+ is reported to be ideal.

Gellan gum hydrogels are transparent hence serve the purpose of inspecting the wound bed during the healing very well. In addition, gellan gum can potentially be applied as in situ gelling liquid or as a hydrogel sheet. Gellan is required in comparatively very low concentrations, strong hydrogels can be easily formed at polymer concentrations <1% (w/w). Gellan gum is a non-toxic biopolymer and also serves to deliver antimicrobial drugs at wound site (Cencetti et al. 2012).

Collagen

Most of the connective tissues show an inherent presence of collagen structurally and hence justify abundance of collagen protein in the mammals. Along with profuse commercial use of collagen as strings for instruments, glues and pharmaceutical capsules, collagen is also being progressively used for WH and TE in biomedical fields. Collagen in nature has many different structural confirmations (Kadler et al. 2008). Fibrous collagens (Collagen Type I, II, III, IV and V) have the ability to form collagen based fibrillar packing molecules together side by side ECM. Owing to its natural origin collagen is the ideal material for WH application. During wound treatment the immune system does not consider it as foreign entity thus drastically reducing chances of rejection in patient (Ruszczak 2003). Collagen fibrils promote cell proliferation and differentiation. High porosity collagen sponges with when used as wound dressing material provide a base during WH. Collagen sponge when applied to burnt area adheres to the skin and provides moist environment. It prevents bacterial infection by cutting the contact between wounded area and external environment. Scaffold is loaded with fibroblasts, which migrates into the wound and proliferates. The sponge, due to its porosity allows invasion of inflammatory cells aiding to faster healing and regeneration of skin tissues. Collagen sponges are

ideal for implantation because they subsequently start degrading, depending upon the cross-linking level, they degrade in an average 3–6 weeks' time period allowing space for replacement with native fibroblast synthesized from type I collagen (Chattopadhyay and Raines 2014). Hydrogels that are collagen-based are used in delivering wound healing agents such as growth factors, anesthetics, various drugs and cells (e.g., fibroblasts). Collagen-based hydrogels loaded with mesenchymal stem cells enhances WH (Rustad et al. 2012). Besides an important wound dressing's material, collagen can be an ideal material for developing tissue engineered substitute for skin. Type I collagen with its biocompatibility shows promising strength to substitute skin directly. Currently, collagen has wide application in WH but it has much more potential to be developed as tissue damage repair material as well as for tissue replacement.

With an ageing population and ever-increasing diabetes propensity within the population, demand for innovative wound healing treatments is expected to skyrocket in the coming times. An increase in demand for a more biologically driven, regenerative medicinal approach in WH, in particular, opens the door to the development of complex biopolymer products that combine medicines, proteins, and cells.

Biopolymers Used in Biomedical Sensors and Implants

Nowadays, Biopolymer based implants and sensors (Fig. 10) are being utilized majorly due to their flexibility, biocompatibility, conductivity, and cost-effective fabrication processes. Biosensors are designed to monitor physiological functioning post-surgery or progressive diseases on the basis of altered homeostatic mechanisms (Alvarez and Lechuga 2010). On the other hand, for restoring and mimicking a damaged organ or organ-part, diagnosis, screening, and treatment of various medical conditions, implants are introduced into the body entirely or partially (Rebelo et al. 2017).

Biopolymer-Based Biosensors

Biosensor is an analytical device which converts the biological signals into electrical/electronic signals. Biosensor contains bio-sensing element as shown in Fig. 11, which senses or detects the concentration or presence of target analyte. This sensing element passes the biological signal to the transducer element which further converts the signal into quantifiable as well as measurable signals such as electrochemical, electrical, electronic, optical, and piezoelectric signals. Appropriate sensors should have certain requirements for instance: (1) Functional and structural stability of the device, (2) Biocompatibility, and (3) Cytocompatibility. Sensing element is usually made up of metal nanoparticle, biopolymers, and carbon-based nanomaterials; among which biopolymers are proven to be the most suitable material till date (Ashammakhi et al. 2021).

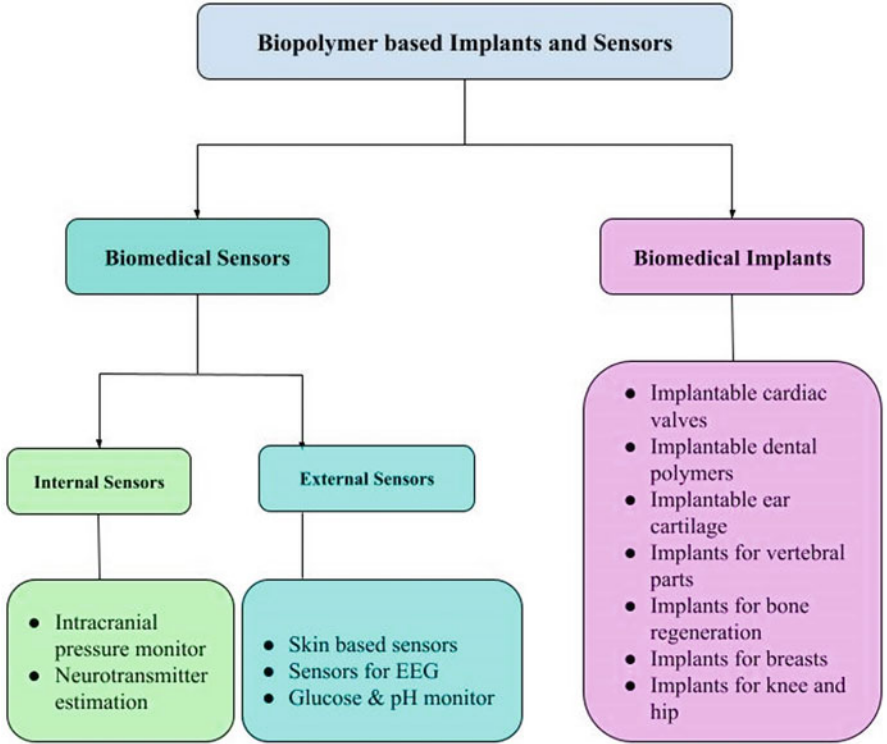


Fig. 10 Flow chart showing biopolymer-based implants and sensors for biomedical applications

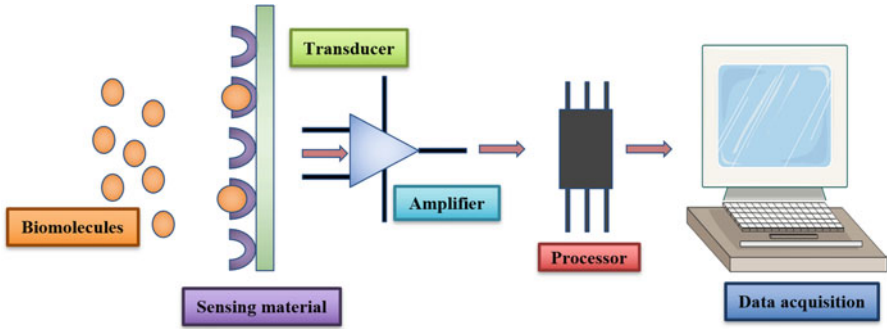


Fig. 11 Basic elements of a Biosensor

Internal Biosensors

Internal biosensors reside within the body for monitoring various physiological conditions. But removal of such internal sensors can be painful, toxic, and expensive procedure. Biopolymers are of exceptional interest for fabricating internal sensors due to their swelling and shrinking capacity, degradability, elasticity, biocompatibility, and their ion exchange capacity than the other materials. It is important for biodegradable sensors to have a protective layer to isolate the internal components of the device from body fluids as they are readily dissolvable (Korostynska et al. 2010).

Biosensors to Monitor Intracranial Pressure

It is extremely important to monitor intracranial pressure for the treatment of stroke, traumatic brain injury, and high blood pressure. A biodegradable sensor, made up of silicon nanomembrane embedded on PLGA was designed to monitor intracranial pressure which progressively disappears as a result of metabolic processes and hydrolysis. This sensor can successfully sense pH, fluid flow, thermal characteristics, and motion (Kang et al. 2016). Apart from that, conductive silk fibroin hydrogel (CSFH) based wearable sensors are fabricated for sensing pressure as well as strain. Through the remotely triggered decomposition mechanism, CSFH based sensors can be degraded. These sensors are reported for having an excellent biocompatibility, mechanical robustness, controlled degradation, and outstanding sensing performance (Zhang et al. 2020).

Biosensors to Detect Neurotransmitter Levels

Neurotransmitters are known to transport chemical signals inside the entire nervous system (NS). Disruption in neurotransmitter signals is detected during neurodegenerative diseases like Parkinson's disease, Alzheimer's disease, schizophrenia, and so on. Monitoring of neurotransmitter level during such neurological condition remains extremely important. Earlier researchers fabricated Poly (o-phenylenediamine) based device to monitor dopamine levels. Since it is a synthetic non-biodegradable polymer-based device, its removal is mandated post-implantation surgeries (Chauhan et al. 2017). Thereafter, PCL based biodegradable biosensors were devised to electrochemically detect dopamine levels patient affected with schizophrenia, Parkinsonism and Attention-deficit/hyperactivity disorder (ADHD) (Leone et al. 2021). Moreover, micro-sensors that can detect glutamate and dopamine levels was fabricated using over oxidized polypyrrole (OPPy) (T. T. C. Tseng and Monbouquette 2012).

External Biosensors

Such biosensors are attached to the body surface externally to sense the physiological and homeostatic information; this information is being sent to the transducer and then to the display. These non-invasive sensors are widely being used for real time

monitoring of wearer's physiological parameters such as pressure, tactile senses, electrical impulses, pH, and glucose levels. For fabricating external biosensors, biopolymers are of major interest due to their ion exchange capacity, durability, biocompatibility, high conductivity, good sensitivity, and stability.

Skin-Based Sensors

It is important to measure pressure, pain and tactile senses in severe medical conditions such as paralysis. Polyvinylidene fluoride (PVDF) based sensors detect pressure and tactile senses as well as blood flow (Fig. 12). Such sensors can work piezoelectrically, i.e., can interchange mechanical energy and electrical energy. Anyhow, PVDF sensors are complex to be fabricated, have low thermal stability as well as expensive. However, further research can be done to avoid such shortcomings (Yiheng Qin et al. 2014).

Sensors for Electroencephalography

Electroencephalogram (EEG) is used in diagnosing altered brain activity in brain related disorders especially in epilepsy. Standard EEG electrodes can detect the signals for an hour. Hence, sensors that can continuously monitor the signals are more appropriate. Alginate based hydrogel sensors are being used for rapid EEG monitoring. Such hydrogel enhances the electrode sensitivity and remains easy to apply and cleanse post-recording. However, hydrogel-based sensors require pre-preparation of hydrogel application over the scalp skin for its usage and it remains the major impediment to the usage of this devise (Cui et al. 2021). Apart from hydrogel, biopolymer based dry electrodes are also designed for the application of EEG. In the dry electrodes (Fig. 13) the hydrating solution loaded in polyurethane foam passes through the Polycarbonate wick. The hydrating solution contains normal saline (for electrical conductivity), tween 80 (for improving permeation of skin), propylene glycol (interacts with lipid layer) and skin irritation avoiding solution. Polycarbonate wick aids to deliver a controlled amount of hydrating solution at the scalp site. Whereas, the silver wire is a signal transduction element, which is directly in contact with the hydrating solution. The whole structure containing Polycarbonate wick as well as PU is encased into a silicone tube from

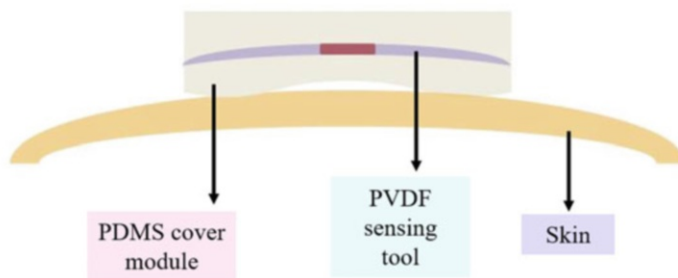


Fig. 12 Schematic image of tactile and pressure sensor

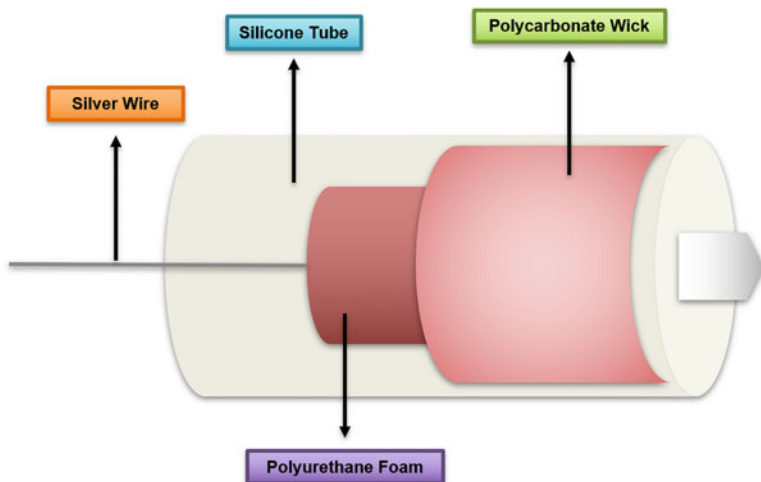


Fig. 13 Schematic image of dry electrode fabricated using biopolymers for EEG applications

which the silver wire exits from the rear end. As compared to our preliminary EEG electrodes, dry electrodes are more comfortable and less time consuming. Moreover, since no post examination cleaning is required, due to which the chances of skin allergies get minimized (Pedrosa et al. 2018).

Sensors for Glucose and pH Monitoring

Glycemic fluctuations, as a part of severe health conditions like diabetes and hypoglycemia needs to be monitored. An implication of such instability becomes critical when the fluctuations are more frequent and usually goes un-noticed especially during pancreatitis, stroke, severe microbial infections, and pregnancy. Currently glucose-based sensors are available in the market. This biosensor includes sensing elements made-up of Glucose oxidase incorporated with P-o-PD which is a semi-conductive polymer, over the electrodes. Between glucose present in the body and glucose oxidase, catalytic enzymatic reaction takes place which produce hydrogen peroxide. Hence, its response depends on the hydrogen peroxide's electrochemical oxidation. Sensing elements detect glucose concentrations which are displayed on the monitor as result of an enzymatic reaction (Korostynska et al. 2010). Kownacka and team designed a tear-based biosensors which can accurately measure glucose concentration using tear. This polysaccharide-based hydrogel coated device can be worn beneath the inferior conjunctival fornix (lower eyelid) which has been proven to be non-painful and non-invasive (Kownacka et al. 2018).

The pH monitoring gains importance in medical condition that requires frequent pH monitoring, such as in deep wound healing. For monitoring pH, biopolymeric polyaniline film (PANI) based pH sensor is devised. Here pencil graphite electrode (PGE) coated with PANI is being used. This pH biosensor has shown high stability, sensitivity, reproducibility with precision (Alam et al. 2018). Recently in 2021 attempts were made to monitor pH and neurometabolic lactate alterations. Here

potentiometric biosensors respond to pH while amperometric biosensors are lactate detectors. Potentiometric biosensors use 5% graphite into conductive polyethylene and micro-fluidic channels whereas lactate-based electrode sensors were able to detect neuro-metabolic transient changes in lactate concentrations in the injured brain mouse model. These sensors are under clinical trials (Booth et al. 2021).

Biopolymer-Based Implants

At the end of the 1950s, implantable medical devices came into the picture. By the early 1960s, active radio transmitting units coated with metal, glass, or ceramic were developed for body cavities such as gastrointestinal tract as well as electronically implantable heart valves were available. Anyhow, removal of such implants requires another surgery. Nowadays biopolymer-based implants are available since they degrade in the presence of oxygen and nitrogen atoms which further lowers the requirement which does away for the need of secondary surgery (Rebelo et al. 2017).

Cardiac Valves

It is reported that nearly 1.3 million individuals suffer from faulty or diseased heart valves globally (Fig. 14). Such dysfunctional valves can cause stenosis (hindrance in the blood flow), blood leakage, or both and are in dire need for replacement. In the 1960s mechanical and bioprosthetic valves were invented to address these

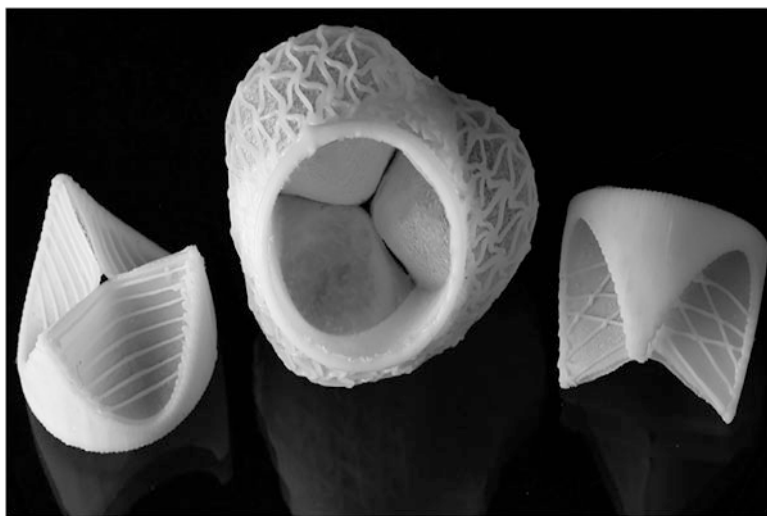


Fig. 14 Silicone based heart valves- with permission © 2019 Elsevier Inc. (Coulter et al. 2019)

osteolysis and high biocompatibility. Furthermore, PEEK implants showed no reported cytotoxicity or carcinogenicity is yet to be reported (Al-Rabab'ah et al. 2019). PEG-like coating has been used on the titanium dental implant to reduce the chances of bacterial adhesion due to their antifouling properties (Buxadera-Palomero et al. 2020).

Implantable Ear Cartilage

Congenital anomalies or traumatic injuries are often the causes of cartilage damage, which may be surgically repairable. Autologous costal cartilage as a graft material is being used for the reconstruction yet, it is limitedly available and gives insufficient measurements. To overcome such limitations biopolymers such as hyaluronic acid, fibrin, silk, collagen is being used. Promising results were seen using benzyl ester of hyaluronic acid in auricular implant, which aids in tissue regeneration like hyaline cartilage within a month period (Aigner et al. 1998). Fibrin-based hydrogel assistance improves the formation of tissue along with cartilaginous microscopic appearance. Injectable scaffold can be created using fibrin-based hydrogel which are less invasive (Balakrishnan and Banerjee 2011). Study conducted at Imperial College London could successfully form cochlear implants using an array of electrode made up of shape memory polymer (SMP) which is fabricated using polylactide (PLA), polytetrafluoroethylene (PFTE), and ethylene-vinyl acetate (EVA) (Fig. 16). SMP uses body temperature for curling up inside the cochlea. The implant converts sounds into the electrical impulses which are further sent to the vestibulocochlear nerve inside the damaged ear. However, inability to absorb moisture inside the ear is sited to be a disadvantage for which further research can be taken up to address this challenge (Bautista-Salinas et al. 2022).

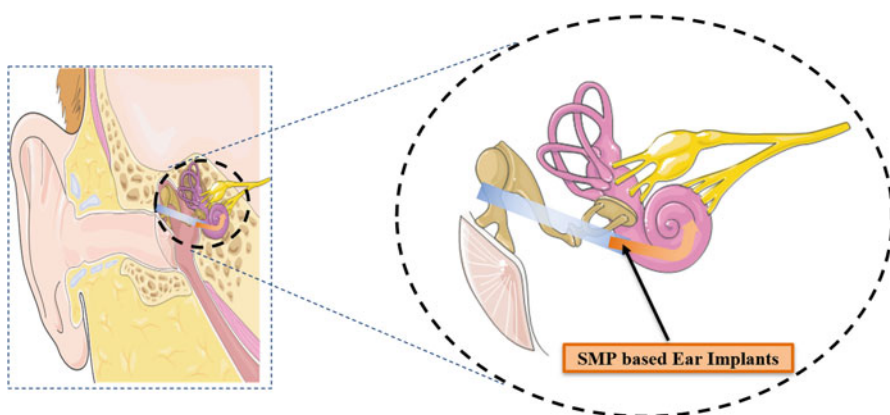


Fig. 16 Schematic image of SMP based ear implants

Biopolymers for Treating Vertebral Parts

The spinal cord is an essential part of our NS which assists in conduction of somatic activities. Its weight bearing capacity and flexibility is often compromised due to age or injury. This calls in for medical implants which can treat the spinal injuries or help to recover the fractured spine. Fibronectin is made of three different folded molecules: Type I, Type II, and Type III. These fibronectin bio materials in the form of cables or mats have been developed to treat injured spinal cord and to repair peripheral nerves. Collagen coated implants and gel matrices are found to be highly permeable, promote glial migration, and help in spinal regeneration (Tabesh et al. 2009). PEEK has become a popular biomaterial for fabricating spinal implants. The *in vivo* studies involving PEEK rod (Fig. 17) demonstrates its excellence as resistor in implant degradation procedure. Currently, such rods are available commercially for lumbar fusion. The radiolucent property of PEEK rods though prevents its observation by x-ray detection (Kurtz 2012). PEEK based implants are widely accepted for fusing the vertebrae together as it provides a significant relief to the patient. PEEK cages (interbody fusion cages) are used for bone fusion, these cages though biocompatible and flexible, can cause mild inflammation in implanted tissue (Kersten et al. 2015).

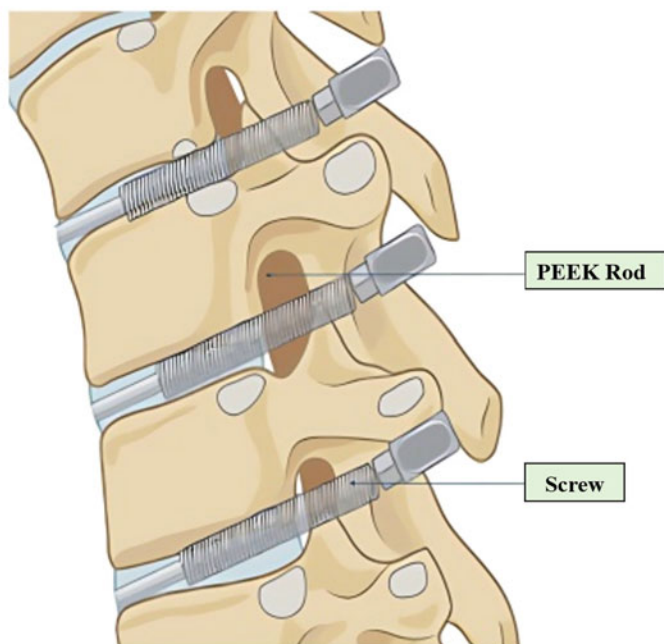


Fig. 17 Schematic image of PEEK rod (Image created in Biorender)

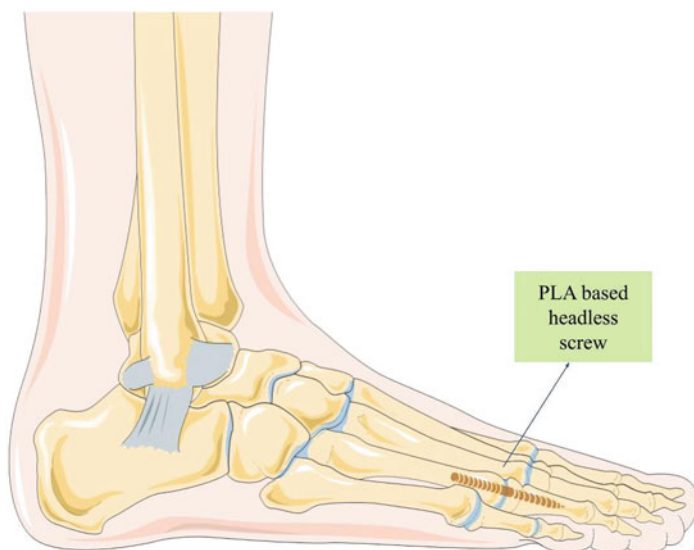


Fig. 18 Schematic image of PLA based headless mini screw

Implants for Bone Repair and Regeneration

Severe accidents often lead to irreparable bone damage. In such grave conditions, medical practitioners often resort to implant materials which can aid in bone repair. Silk fibroin bone implants provides flexibility, high mechanical support, thermal stability and can be subjected to chemical modification easily. However, their biocompatibility is yet to be tested in humans (Meinel et al. 2005). Use of natural polymeric materials like chitosan, collagen often leads to early polymeric degradation to the bones, which may need 4–6 months of healing period (Wu et al. 2018). In 2014, Chitosan/alginate/hydroxyapatite (CAH/ B2) scaffolds were used as implants for bone regeneration, the in-vivo study demonstrated enhanced bone healing in rats and thereby concluded that CAH/B2 scaffolds could enhance osteogenic bone morphogenetic protein-2 delivery (He et al. 2014). PLA-based screws (Fig. 18) are now available for graft, bone, tissue, vertebral fixation, and ligament reconstruction. Unlike titanium screws, PLA-based screws reduces the need for subsequent operation for its removal. However, it is noted that bones and tissues often takes time to fill up the hole formed by polymer degeneration (Tappa et al. 2019).

Breast Implants

By the end of 2020, WHO reported approximately 7.8 million women were suffering from breast cancer, of which majority of them undergo mastectomy. Cosmetic surgery using breast implants can bring aesthetic relief to such patients (Fig. 19). Polyurethane foam-based breast implants are reported to cause to less irritation,

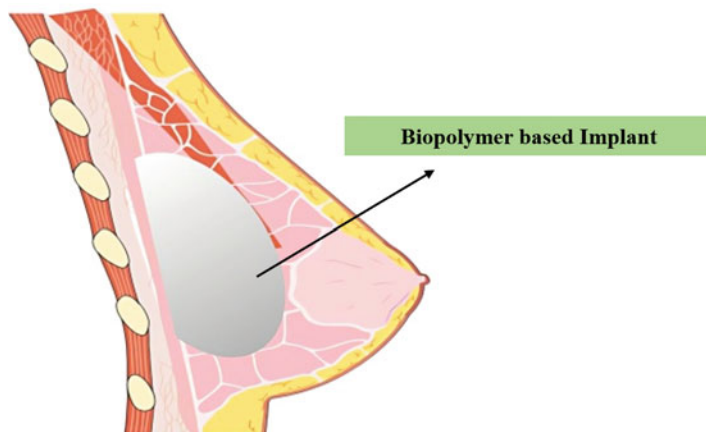


Fig. 19 Schematic image of implanted breast

sensitization, and toxicity with almost meager to no immunological complications. These implants have inherent limitations of untimely implant rupture resulting in sticky sensation and skin rashes (Handel and Gutierrez 2006). Currently, silicone based pre-filled silicone gel breasts are widely preferred. However, cases of breast pain, infection as well as implant rupture have been reported. Nevertheless, most importantly such implants mimic human fat accurately, thus they feel more natural in morphology (Balk et al. 2016).

Knee and Hip Implants

To relieve the severe osteoarthritic pain, patients often undergo knee replacement surgery. Standard knee replacement surgery includes metals such as titanium or cobalt based prosthetics. However biopolymer-based implants are now designed and claimed to be more convenient. PEEK-based knee implants are reported to be most reliable due to their better mechanical support and biocompatibility. Recent studies claim PEEK to be more efficient than Ultra High Molecular Weight Polyethylene (UHMWPE) for overall knee replacement surgery (Sandeep Kumar et al. 2020). UHMWPE has high impact strength, low friction quality, and excellently wear resistance. Similarly, hip implants offer relief from arthritis, necrosis, and hip joint damage. Currently, UHMWPE cobalt-based alloys are commonly used for production of hip joint implant, especially for overweight patients (Merola and Affatato 2019).

Conclusion

The need for breakthrough biomedical applications and treatments is predicted to grow in the coming decade as the population ages. The demand for a more biologically driven, regenerative medicinal approach in the field of TE, drug delivery,

wound healing, biomedical implants, as well as sensors in particular has surged. This provides excellent opportunities for biopolymer research to unravel beneficial properties which may help in the development and delivery of treatments in the coming future.

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Nanocellulose from *Cladophora*

31

Applications

P. V. Sandhya, M. Nishaf Naseeha Farsana, and K. S. Femina

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Abstract

Cellulose is a unique biopolymer having distinctive structure and exceptional qualities beneficial for numerous industrial, environmental, medicinal, and pharmaceutical applications. *Cladophora* nanocellulose, extracted from *Cladophora* green algae, has unmatched advantages over those celluloses derived from other plant sources. Unique features of *Cladophora*-derived cellulose include excellent crystallinity (>95%), low moisture adsorption capacity, better solution processability, high porosity in the mesoporous range, and larger specific surface area. Due to its rich surface functionality, it can be easily modified by simple chemical reactions. Numerous *Cladophora* nanocellulose derivatives and composites have been reported, and their applications have been explored in diverse fields, including biomedical, environmental remediation, energy storage, batteries, etc. The pore size's tenability could be applied as an immunosorbent, hemodialysis, and

P. V. Sandhya · M. N. Naseeha Farsana · K. S. Femina (✉)

Post-Graduate and Research Department of Chemistry, Maharajas College, Ernakulam, India

e-mail: femina@maharajas.ac.in

viral filter. This chapter comprehensively describes the unique physicochemical features and applications of Cladophora nanocellulose and its composites.

Keywords

Paper electrode · Adsorption · Size exclusion · Capacitor · Mesoporous · Composite · Polyaniline · Polypyrrole

Introduction

Cellulose is one of the most promising green materials for a bio-based future and sustainable economy due to its natural abundance, fibrous structure, mechanical flexibility, ease of chemical modification, biocompatibility, and renewability. Cellulose made from wood or bacteria has dominated the industrial cellulose market and has been developed to provide various innovative materials for energy storage, environmental, and biotechnology applications (Lavanya et al. 2015).

Marine algal biomass is rich in biopolymers like cellulose. In 1885, algal cellulose was extracted and described for the first time, and later, cellulose was extracted from numerous algal species. Cellulose can conveniently be extracted from marine algal biomass and used to prepare composites or support matrices for various applications and new industries. As a renewable energy source, using cellulose-based products as an alternative makes use of synthetic polymers or materials, which may conserve energy and foster economy while addressing environmental issues. Cellulose isolated from Cladophora, a green alga designated as Cladophora cellulose, has unmatched benefits over cellulose obtained from other methods like wood or bacterial celluloses due to its high crystallinity (>95%), low moisture absorption capacity, superior solution processability, high porosity especially in the mesoporous region, and larger specific surface area (Baghel et al. 2021; Lavanya et al. 2015; Romruen et al. 2022; Siddhanta et al. 2011; Zhou et al. 2019). This chapter comprehensively describes the unique physicochemical features of Cladophora nanocellulose-based materials and their applications in diverse fields.

Nanocellulose from Cladophora: Extraction and Properties

Cellulose is the most prevalent biopolymer found in the cell walls of all plant cells. Apart from plants, cellulose can be obtained from algae, certain bacteria, and fungi. The chemical formula of cellulose is $(C_6H_{10}O_5)_n$. There are thousands of D-glucose subunits in cellulose. The D-glucopyranose units are held together by a covalent bond between the OH group of C-4 and C-1 carbon atoms (Fig. 1). Unlike other polysaccharides, the D-glucose molecules in cellulose have a beta orientation, with the anomeric carbon's hydroxyl group pointed above the plane of the glucose ring. The hydroxyl groups of the remaining carbon atoms are pointed below the ring plane. Thus, in cellulose chains, every alternate glucose molecule is reversed. Unlike

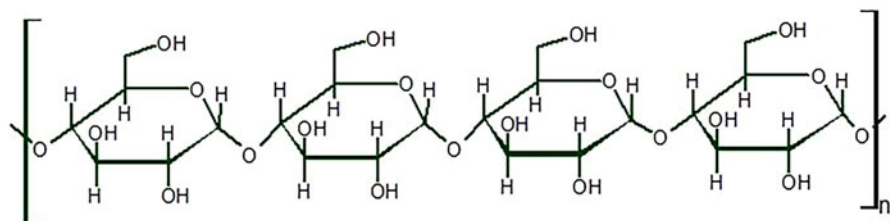


Fig. 1 Chemical structure of cellulose

starch or glycogen, the polymeric chains of glucose are organized linearly and do not undergo coiling, helix formation, or branching. Instead, these chains are parallel to one another. These chains are stacked in parallel to produce microfibrils. The tensile strength of cellulose microfibrils is due to the strong hydrogen bonds between the individual chains in cellulose microfibrils. The shape, length, and diameter of cellulose structures in nature vary depending on the source and process through which they are extracted. The donor group, OH, is responsible for chirality, hydrophilicity, degradability, and chemical flexibility – crystallinity in cellulose results from its strong hydrogen bonding. Cellulose's unique structural features come from the fact that, unlike most polysaccharides, it can maintain a semi-crystalline state of aggregation even in water (Baghel et al. 2021; Mihranyan 2011; Steven et al. 2021).

Most of the cellulose used in the industry comes from wood pulp and cotton. To get cellulose out of such lignocellulosic feedstock as wood or cotton, lignin needs to be removed, and the lignin removing process requires necessary chemical treatment and may cause partial cellulose degradation. Delignification is an energy-consuming process, and cellulose thus obtained may be contaminated with chemicals or by-products (Mihranyan 2011).

In addition to lignocellulosic biomass, cellulose is present in the cell walls of marine biomass, including macroalgae. Cellulose derived from macroalgae has attracted considerable interest recently due to its several advantages over cellulose derived from terrestrial biomass. The lack of lignin in macroalgae results in purer cellulose fractions that may be more appropriate for biomedical applications than lignocellulose. Additionally, the absence of lignin enables cellulose extraction under milder conditions, resulting in less degraded cellulose segments.

Macroalgae is a low-cost source of biomass, which multiplies, and cultivation does not require irrigation or arable land areas. Furthermore, macroalgae farming does not necessitate the use of fertilizers, hence preventing coastal eutrophication. It has been demonstrated that cellulose can be successfully extracted from brown, green, and even red macroalgae. The total amount of cellulose that makes up a plant's dry weight can range from around 1% to 15% (w/weight), depending on the species. The amount of cellulose present is prone to seasonal shifts and is extremely sensitive to the degree to which the biomass has matured (Mihranyan 2011; Zhou et al. 2019).

Cladophora is a filamentous green alga that belongs to Chlorophyta, commonly known as Mekong Weed (Fig. 2). It is a large genus and includes 183 taxonomically

Fig. 2 Photograph of *Cladophora* green alga. (Inset: Magnified image)



accepted algal species. It possesses thick walls without a mucilaginous sheath, dichotomously branched thallus with narrow tips and rough texture. Cells are large, cylindrical, multinucleated with reticulate chloroplast and numerous pyrenoids. The bright green color of *Cladophora* reflects the presence of pigment chlorophyll a, b, β -carotene, and xanthophylls. It contains chlorophyll a and b in the same ratio as in higher plants. *Cladophora* naturally grows on surfaces of substrates using hold fast. It often detaches from substrates and accumulates along shores, forming thick mats. High light intensity and nutrient-rich neutral or slightly alkaline water are needed for their growth. Their metabolism and morphologies are significantly affected by temperature, water currents, and wave action. *Cladophora* usually exhibits an isomorphic diplohaplontic type of life cycle and reproduces by vegetative, asexual, and sexual modes of reproduction. *Cladophora* can grow in single, bi-, and polyculture systems for biomass production (Munir et al. 2019; Stabili et al. 2014; Steven et al. 2021).

Cellulose is present in the cell walls of *Cladophora* green algae, and the properties and content of cellulose obtained may vary according to species. The cellulose content of several *Cladophora* species was analyzed, and the studies revealed that the cellulose content varied from 5% to 34% by dry weight. However, it was possible to recover cellulose as high as 45% on a dry weight from residual seaweed biomass, comparable to the cellulose content of higher plants. The findings suggested that biomass from *Cladophora* algae could be employed in industrial cellulose production. The cellulose fibers were conveniently extracted from the fresh *Cladophora* biomass and dried residual biomass. In addition to its economic benefit, the extraction of cellulose fibers from *Cladophora* algal sources can be treated as a method of environmental restoration. Using algal biomass to extract cellulose fibers helps reduce the damage caused by these algae's excessive and undesirable growth to the marine ecology (Brett 2000; Imai and Sugiyama 1998; Mihranyan 2011).

Primarily cellulose is taken from the dry biomass of entire seaweed. The extraction procedure is analogous to that used in the timber industry, although under milder conditions. Mihranyan et al. prepared exceptionally crystalline *Cladophora* cellulose by exposing bleached *Cladophora* green algae to acid hydrolysis and keeping it overnight (Ek et al. 1998; Mihranyan 2011). The typical cellulose extraction process from residual biomass consists of a series of steps and is illustrated in Fig. 3. The initial step involves bleaching dried seaweed biomass with sodium chlorite (NaClO_2) in an acetate buffer at 60 °C for about 3–6 h, then neutralizing the material by washing it with distilled water. Then, it was treated with 0.5 M NaOH at 60 °C for 6–12 h, followed by washing till pH became 7. The alkali-treated material was then boiled in 5% HCl (v/v), followed by room-temperature incubation. It was then



Fig. 3 Flow chart showing extraction of cellulose from *Cladophora* biomass

washed till the filtrate became neutral and dried to obtain the cellulose. The cellulose thus obtained is usually mesoporous or microporous and amorphous (Romruen et al. 2022).

Nanocelluloses are exceptional and promising natural materials among cellulose-derived goods. Based on structural features and origin, nanocellulose is classified into three types: (1) nanocrystalline cellulose, (2) cellulose nanofiber, and (3) bacterial cellulose. The size and crystallinity of nanocellulose are the most prominent properties. Nanocellulose's lateral dimensions range from 5 to 20 nm, and its longitudinal dimensions are a few microns. Nanocellulose is a light solid made of nanosized cellulose fibrils. This pseudo-plastic substance has the properties of thick fluids or gels. Due to its larger surface area and nanoscale size, the nanoform of cellulose is superior to cellulosic microfibrils (Mihiranyan 2011; Zhou et al. 2019).

Cladophora nanocellulose was obtained by hydrolyzing the chemical-sensitive amorphous portion of cellulose microfibrils in a controlled manner. A strong acid like sulfuric acid is often used for the hydrolysis process that breaks down amorphous cellulose to get nanocellulose (Steven et al. 2021). Siddhant et al. presented an alternative approach in which *Cladophora* green algae was defatted by repeated extraction in methanol for a longer time at room temperature, followed by treatment with NaOH. The defatted dried algae are further bleached by soaking in an acetate buffer, and the defatted dried algae are resuspended in HCl and cooked. This cellulose could be spread using a variety of techniques (Baghel et al. 2021; Romruen et al. 2022; Siddhanta et al. 2009, 2011; Zhou et al. 2019). Cladophora nanocellulose can be stored as a powder easily dispersed in water to produce a homogeneous hydrogel or suspension, which is attributed to its increased crystallinity and nanofibrous shape. Cladophora nanocellulose is strong, flexible, and light. It is biocompatible, bio-expandable, and has a low toxicity level. The cellulose surface can easily be modified because of many hydroxyl groups (Igor et al. 2016; Mihiranyan 2011).

The structure of Cladophora nanocellulose is more similar to nanocellulose produced by bacteria than to the corresponding nanocellulose from wood or cotton. Cellulose derived from Cladophora mostly belongs to polymorph I, characterized by parallel strands rather than intersheet hydrogen bonding. Crystal structural changes induce differences in cellulose I and II. Cellulose I is a mix of two different types of cellulose, called cellulose I α and cellulose I β . Like bacterial cellulose, Cladophora algal celluloses have many triclinic I α allomorphs, while wood-derived celluloses mainly comprise I β allomorphs. The cellulose I α allomorph is more likely to be broken down by enzymes, while cellulose found in cotton, which is high in the I β allomorph of cellulose, has a lot of disordered and is prone to chemical reactions. The presence of an amorphous fraction mainly decides the overall reactivity of the cellulose substrate. However, for nanocellulose obtained by the hydrolysis of Cladophora cellulose, the crystallinity is typical >95%, which is higher than that of bacterial cellulose and significantly higher than that of cellulose derived from wood or cotton. Because of this unique structure and high crystallinity, Cladophora nanocellulose is less hygroscopic than cellulose derived from other sources. The available surface area for water molecules in cellulose decreases steadily with increasing crystallinity (Mihiranyan 2011).

Cladophora nanocellulose retains its capacity to disperse in water and create stable dispersions through ultrasonication due to its nanoscale properties and low hornification level. These dispersions offer an efficient method for organizing and processing nanocellulose into a wide range of bulk products (Suciyati et al. 2021; Xiang et al. 2016).

Rheological qualities reveal a material's microstructure and the degree and pattern of material deformation under stress. Rheology analyzes fluid mechanics of a sample like stress, relaxation, viscosity etc. Recently, Mihranyan et al. examined that *Cladophora* cellulose powder could be a viable alternative to dispersible cellulose grades. They dispersed *Cladophora* cellulose in the liquid phase via high-frequency sonication to give complex, semisolid products with viscoelastic qualities based on oscillating frequency. According to the study, a 0.2% dispersion of *Cladophora* cellulose exhibits the properties of a fluid dispersion ($\tan \delta \gg 0.176$) rather than a true gel (Mihranyan et al. 2007).

Furthermore, it was shown that the material's elastic and viscous modulus improved with increasing cellulose concentration, and the gel strength declined as the concentration of carboxymethylcellulose increased. This study provides evidence that *Cladophora* cellulose powder may be a suitable replacement for commercially available dispersible cellulose grades. Its utilization may even provide benefits over already utilized solutions in circumstances where a more potent gelling action is required and when adding a minor solid phase into the central dispersive system is mandated (Mihranyan et al. 2007).

By accumulating interfiber gaps, *Cladophora* cellulose is highly porous (pore volume $0.55 \text{ m}^3 \text{ g}^{-1}$) and has a high specific surface area ($95 \text{ m}^2 \text{ g}^{-1}$) which was corroborated by BET surface area measurements. Good mesoporosity and non-swelling upon contact with water are advantageous for battery separator or water purification applications. Due to the abundance of hydroxy groups on the surface of cellulose nanofibrils, their surface can be easily modifiable to produce functional cellulose derivatives. Due to the ease of chemical modification, *Cladophora* nanocellulose offers other choices for less commonly utilized reactions, such as aldehydes by periodate oxidation, diamines via reductive amination of 2,3-dialdehydes, and sulfonyl via a sulfonation reaction, while maintaining the original nanofiber morphology. Being highly crystalline, *Cladophora* nanocellulose can withstand strong reaction conditions like high temperature and pressure (Rocha, Hattori, et al. 2018; Steven et al. 2021).

Mihranyan et al. coated *Cladophora* cellulose and prepared two different routes with conducting PPy via oxidative chemical polymerization. They could observe that coating pure *Cladophora* cellulose nanofibers with PPy shifted the pore size distribution to higher, resulting in composites with an open, loosely packed structure with a large surface area. The composites' electroactivity depended on the composite's total porosity. Compared to clean nanocellulose samples, the composites' mechanical strength has been reduced due to the breakdown in H-bonding between neighboring cellulose fibers. However, the nanocellulose-PPy nanocomposites obtained by adding bare nanocellulose fibers to PPy showed improved mechanical properties (Mihranyan et al. 2012).

Cellulose is a good choice for electrical insulator applications due to its unique properties such as low resistance, wide availability, low cost, high mechanical strength, and thermal stability. However, due to the presence of hydroxyl groups, cellulose can absorb water, and hygroscopicity is the most significant barrier to its utilization in electrical insulator applications. For electrical insulator application, the moisture content of cellulose should be 0.5% or less. Otherwise, the paper must be treated and kept dry, which is an extensive, time-consuming, and costly procedure. According to the results of He pycnometry, N₂ sorption, and SEM studies reported by le Bras and coworkers, the overall porosity of Cladophora cellulose is around 44%, which is much higher than that of wood-derived cellulose. They could also observe that Cladophora cellulose sheets are substantially more permeable. The high porosity and residual air in Cladophora cellulose paper result in increased dielectric loss. The Cladophora cellulose exhibited a moisture sorption pattern that was characteristic of that of non-hygroscopic mesoporous materials. Water sorption studies confirmed that Cladophora cellulose has a limited moisture sorption capability at low to moderate vapor pressures, while at high relative vapor pressures, condensation in numerous pores causes a fast surge in adsorbed water. The specific surface area values determined via N₂ and water sorption studies for Cladophora cellulose are similar, while that for wood cellulose was different. This finding suggests that, unlike wood, in which cellulose penetrates the solid mass in the disordered regions, the water sorption in Cladophora cellulose takes place only on the surface of fibrils due to the high crystallinity of the cellulose. It was observed that porosity influences mechanical properties, and Cladophora cellulose has lower tensile strength, tensile index, Young's modulus, and yield stress compared to wood-derived cellulose. However, the tensile strength per relative density was the same in both samples, indicating that the inherent strength of cellulose fibers is stable. Based on the studies on dielectric behavior, it was proposed that the increase in conductivity and dielectric loss increased with an increase in moisture content. The adsorbed water molecules on the nanofibrils' surface of Cladophora cellulose are less tightly bonded and can create a channel for ions and increase ionic mobility. The low moisture sorption properties, conductivity, and dielectric parameters of Cladophora cellulose are very suitable for developing novel high-power paper insulators (le Bras et al. 2015).

Mihranyan et al. established that incorporating nanocellulose could improve the mechanical properties of conductive paper electrodes. They studied the effect of incorporating nanocellulose having different porosity, density, and tensile strength obtained from three Cladophora cellulose sources. Interestingly the tensile strength observed for the composites was much lower than pristine nanocellulose. The low tensile strength of nanocellulose cannot be accounted for by the large pore sizes and higher porosity. Mihranyan et al. argued that the declining mechanical capabilities could be due to H-bond shielding between O-H groups on cellulose nanofiber surfaces. In order to achieve good electrical conductivity, continual polypyrrole (PPy) coating on nanocellulose fibers is needed. Incorporation of PPy via in situ polymerization weakens H-bonding between ordinarily robust nanocellulose sheets within the composite. The study adopted post-synthetic routes for incorporating PPy,

and the mechanical characteristics of nanocellulose-PPy composites were improved to a great extent (Mihrianyan et al. 2008, 2012). The unique physical and chemical properties of *Cladophora* nanocellulose can add new features and improve the performance of nanocellulose-based materials, and these characteristics have garnered significant scientific interest over the past decade. Due to its unique physico-chemical properties, it can be used in many different areas, including composite reinforcement, scaffolds for tissue engineering, biosensors, drug delivery, and electronics.

Applications of *Cladophora* Nanocellulose

Cladophora nanocellulose is particularly valuable because of its unique structure, lightweight, excellent mechanical performance, large surface area, aspect ratio, biocompatibility, wide availability, and the ability to change its surface to increase nano-reinforcement compatibility with other polymers. Both academia and industries are looking into new ways to produce and use nanocellulose, a vital material in developing commercial nanostructured materials. In comparison to carbon fiber and glass fiber, nanocellulose is more economical. There are a wide variety of applications for *Cladophora* nanocellulose. However, most of these applications are in the mechanical, medical, and industrial sectors. Degradable polymer composites often use cellulose as a matrix filler in their composition. Additionally, applications for cellulose can be found in the fields of packaging, tissue engineering, sensors, pharmaceuticals, electronic materials, water filtration, and viral filters. Moreover, cellulose has been utilized in conjunction with carbon nanotubes and conducting polymers as current collectors in Li-ion battery applications and electrode material for supercapacitors. Therefore by using nontoxic, recyclable, carbon neutral, and sustainable *Cladophora* nanocellulose based goods as an alternative for synthetic or petrochemical-based components, most of these industries can lessen their environmental impact.

Environmental Pollution Remediation Applications

Many environmental issues have arisen because of the rapid development of industrialization and urbanization worldwide in recent decades. Water pollution from large-scale industrial production has raised serious concerns about water scarcity and safety. Burning large amounts of fossil fuels have resulted in a significant increase in carbon dioxide emissions, resulting in apparent global warming due to the greenhouse effect. Poisonous gases (SO₂, NO_x, volatile organic compounds (VOCs), and other pollutants in the air) are significant causes of respiratory diseases (Yoon et al. 2008). Despite developing numerous materials and technologies to address these environmental issues, more effective and environmentally sustainable strategies are still required. *Cladophora* nanocellulose can be effectively used for water purification, heavy metal ion adsorption, CO₂ absorption, organic gas

adsorption, and separation as a naturally abundant, sustainable nanomaterial with inherent mesoporosity.

Cladophora Cellulose for Water Purification

Water purification necessitates the rapid and efficient filtration and adsorption of impurities in the water to make it safe to drink. Synthetic polymers (e.g., polyamides) with micro- or mesopores (pore diameter of <2 nm or 2–50 nm, respectively) are the most common materials used in commercial water filtration membranes. Cellulose is a standard and inexpensive filter with a long history. However, when large amounts of water need to be filtered for nanoscale contaminants, ordinary filter paper with micrometer-sized pores is unmanageable (nanoparticles, viruses, etc.) (Thomas et al. 2018). The limited processability of bacterial cellulose nanofibers and the low porosity of wood cellulose limit their use as water filtration membranes. On the other hand, *Cladophora* cellulose membranes with high porosity are promising for water purification (Fig. 4a) (Gustafsson et al. 2018). A simulation of wastewater filtration revealed that *Cladophora* cellulose-based papers could remove nano pollutants (100 nm) with up to 99.99% efficiency at industrial flow rates ($60\text{--}500\text{ L m}^{-2}\text{ h}^{-1}$) (Fig. 4b, c). Furthermore, the study reported that chemically altering *Cladophora* cellulose produces a new class of high-performance adsorbents: surface-functionalized *Cladophora* cellulose beads (Fig. 4d, e) can be used to effectively adsorb metal ions and small organic molecules from water (Fig. 4f) (Ruan et al. 2016).

The cellulose obtained from the cell walls of *Cladophora* algae is insoluble in water; it does not swell as much as cellulose from other familiar sources, suggesting that it has a lower capacity to absorb water (Xiang et al. 2016). This observation points to the highly crystalline nature of the lake-derived *Cladophora* cellulose. If the cellulose has a high degree of crystallinity, water cannot easily pass through the chains despite numerous hydroxyl groups. This finding backs up a study by Mihranyan et al. that claims cellulose absorbs less moisture as its degree of crystallinity rises (Mihranyan, Llagostera, et al. 2004). The quantity of available hydroxyl groups, or in other words, the degree of crystallinity, plays a significant role in how easily water can permeate the structure and cause swelling. Compared to wood cellulose, the *Cladophora* sample's high degree of crystallinity suggests a higher order of the polymer chains and, consequently, a higher surface area. The native cellulose found in terrestrial plants, known as type I cellulose, frequently used in the paper industry, has a relatively small specific area of $1\text{ m}^2/\text{g}$. On the other hand, the cellulose obtained from the cell walls of *Cladophora* sp. green algae is highly crystalline and has a distinctively high surface texture made of numerous intertwined strings, resulting in an aggregate web-like structure with high porosity and a large surface area. It is well known that polymers like polypyrrole (PPy) can effectively wet cellulose fibers, which makes it possible to coat each fiber uniformly. Composites made of cellulose and conductive polymers are desirable because they are entirely recyclable, light in weight, mechanically robust, and quickly produced.

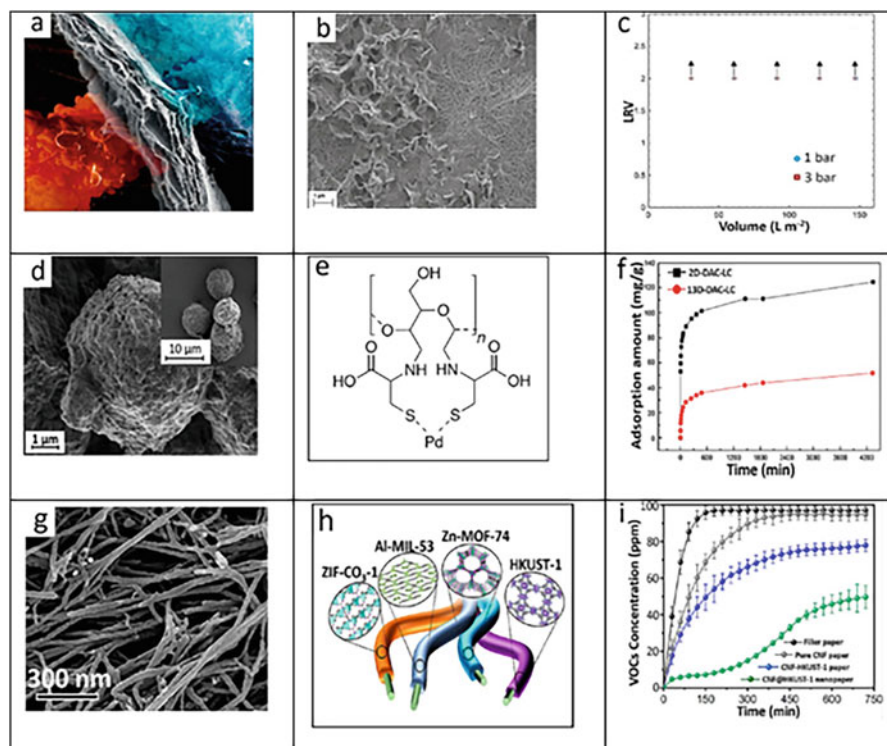


Fig. 4 SEM images of Cladophora cellulose nano paper for water purification (a) cross-sectional and (b) surface. (c) Logarithm reduction values for Cladophora cellulose nano paper filtration of simulated wastewater. (d) SEM image and (e) molecular structure of a cysteine 2,3-dialdehyde-functionalized Cladophora cellulose bead. (f) Functionalized Cladophora cellulose adsorption isotherm for Pd(II). SEM image of Cladophora cellulose@metal organic framework nanofibers (g) and schematic illustration of Cladophora cellulose @ metal organic framework nanofibers (h). (i) The ability of normal filter paper, pure Cladophora cellulose, Cladophora cellulose – metal organic framework, and Cladophora cellulose@ metal organic framework nano papers to filter volatile organic compounds (VOCs). (Gustafsson et al. 2018) (a–c), (d–f) Ref. (Ruan et al. 2016), and (g–i) Ref. (Zhou et al. 2019) were used with permission)

They allow the aniline monomers to adsorb onto the surface of the cellulose fibers before allowing the polymerization agent to pass through, resulting in the polymerization of aniline with the formation of a thin coating on cellulose fibers. By forming an H-bond between the oxygen moieties in cellulose and the aniline N-H groups, cellulose fiber that meets the reaction mixture is coated with a thin layer of PANI. In contrast to the micro- and milli-layers that can be formed by combining the monomer, polymerization agent, and cellulose in a single pot, a nanolayer of polyaniline (PANI) is developed on the cellulose fibers using this method. A PANI coating nanolayer will maintain the cellulose's high surface area. An effective polymerization can be seen from the blue coloration that develops after allowing the polymerization agent to pass through the cellulose with adsorbed aniline. The obtained

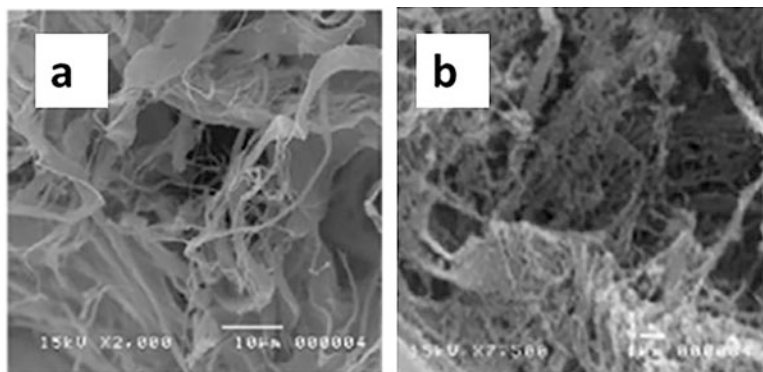


Fig. 5 SEM image of pure Cladophora nanocellulose (a) and PANI-coated Cladophora nanocellulose (b) (Tan et al. 2018)

cellulose-PANI composite is still blue in color, indicating that PANI is still locked in the cellulose fibers, even after washing several times in acetone and sonicating the residue to remove the free PANI. While the SEM images of Cladophora cellulose coated with PANI show all fiber surfaces coated with an amorphous/granular-like coating, indicating uniform morphological modification, the SEM images of pure Cladophora cellulose (Fig. 5a) show intertwining fibers with smooth surfaces. It was confirmed that the cellulose fibers are coated with PANI nano-layers (50 nm) while maintaining their high surface area (Fig. 5b) (Tan et al. 2018).

By comparing the electrical conductivity and resistivity of the composite to those of pure Cladophora cellulose, it is possible to confirm the presence of PANI coating on the cellulose fibers. Using the four-probe conductivity meter, the electrical conductivity of the composite was measured and found to be significantly higher (0.670 S/cm) than that of pure cellulose (0.091 S/cm). The conductive polymer coating's presence is what causes the conductivity to improve. The higher surface area of PANI on Cladophora cellulose can be attributed to the conductivity measured in this study's better performance. This implies that highly conducting composites can be created using conducting component nanolayers. The manufactured cellulose/PANI composite was used to remove toxic environmental metals. Chromium is used in industry, especially in the tanning and plating sectors. Chromium ions are frequently dumped into the river system with wastewater after being used. There are two oxidation states of chromium: Cr (VI) and Cr (III). Due to their unusual redox activity, conducting polymers provide an alternative method for the remediation of Cr(VI). It has been reported that pure PANI films were used before pure PPy films to reduce highly toxic hexavalent chromium ions to less toxic trivalent chromium ions utilizing conducting polymers. When PANI is exposed to hexavalent chromium, the PANI in its reduced form (emeraldine base, EB) will function as a reducing agent. When the Cr (VI) is reduced to Cr, the PANI will subsequently be oxidized to pernigraniline. Redox activity and surface adsorption may work together to remove chromium in the presence of cellulose/PANI composite. The reduction of hexavalent

chromium ion to trivalent chromium by *Cladophora* nanocellulose-PANI composite caused the emeraldine form of PANI to get oxidized to pernigraniline form change in acidic pH. Although PANI (emeraldine form) is not a metal, its reduction potential is comparable to silver's, making it effective at reducing chromium. Surface adsorption may also be a viable alternative mechanism. Positively charged chromium ion gets coordinated through the lone pair electron of nitrogen in the *Cladophora* cellulose-PANI composite. Additionally, electrostatic attraction may happen under acidic conditions between solution anions (chromate and dichromate) and the protonated form of nitrogen atoms in PANI. The study established that using low-cost *Cladophora* cellulose coated with nano-layers of PANI, toxic hexavalent chromium can be removed from wastewater regardless of the mechanism (Camacho et al. 2013; Mhraryan et al. 2012; Razaq et al. 2012). 2,3-Dialdehyde cellulose (DAC) prepared from the *Cladophora* nanocellulose via chemical modification acts as an excellent adsorbent for the metal Pd (II). DAC can be prepared as the first *Cladophora* nanocellulose in water mixed with sodium meta periodate. The periodate-containing reaction mixture was meticulously covered in aluminum foil to protect it from light. For 13 days, the reaction mixture was stirred briskly at room temperature in the dark. After that, ethanol was added, and the DAC was repeatedly washed with water to produce pure DAC. The adsorbent was produced by reductive amination, which functionalized the DAC with natural amino acid cysteine. Plenty of coordination in the functionalized DACs sites makes palladium adsorption effective. The DAC materials with L-cysteine functionalization were then put through Pd(II) ion adsorption and provided a large adsorption capacity (Ruan et al. 2016).

The adsorption of Congo red is a fascinating subject that has received much research. Congo red aqueous solutions turn red above a pH of 5 and blue below that. When the Congo red dye solution of a definite pH is shaken well with the DAC, it was observed that the intensity of the dye is decreased, indicating its adsorption by DAC. The dye can be removed from the solution by penetrating the voids of this nanocrystalline particle derived from *Cladophora*. Similarly, nicotine is also adsorbed into the vacant space of the DAC (C. Q. Ruan et al. 2018).

Remediation of Air Pollution

Selectively adsorption of noxious gases from the air is essential for air purification. The adsorption process requires materials with high specific surface areas and interconnected nanopore structures. Nanopore structure is practically unattainable for pure *Cladophora* cellulose. Studies demonstrated that the pore structures could be tuned by combining metalorganic frameworks with *Cladophora* nanocellulose, and novel crystalline nanoporous materials that can function as efficient adsorbents for removing noxious gases from the polluted air can be developed. It was observed that the natural blending of metal-organic framework particles with *Cladophora* nanocellulose into a composite paper was inefficient due to the poor gas flow between metal-organic framework particles. Using a new nanostructure in which metal-organic frameworks are assembled into continuous nanolayers on *Cladophora*

nanocellulose to form hybrid nanofibers, the effective contact area for the gas can be improved (Zhou et al. 2019). Thus, the prepared hybrid nanofiller achieved high efficiency in VOC separation (air purification). A pyrolysis process involving sodium oxidation, carbonization, and activation was used to convert *Cladophora* nanocellulose into sustainable activated carbons. High surface areas (up to $1241 \text{ m}^2\text{g}^{-1}$) and tunable micropore sizes (pore diameter $<0.8 \text{ nm}$) were found in the activated carbon (Xu et al. 2018). The activated carbon had a high capacity for CO_2 uptake ($5.52 \text{ m mol g}^{-1}$ at 1 bar and 273 K) and high CO_2 -over- N_2 selectivity (Pan et al. 2018).

Biomedical Applications

Biomedical applications of algal cellulose and its derivatives are gaining popularity. The physical, mechanical, chemical, and biological properties of *Cladophora* cellulose are unrivalled by those of synthetic polymers. Hence these biocompatible, nontoxic *Cladophora* cellulose could replace synthetic polymers in several biomedical applications, including tissue engineering and drug delivery. Due to their biocompatibility, biodegradability, low cytotoxicity, high crystallinity, tunable porosity, and rich surface functionality, *Cladophora* cellulose has proved a cost-effective future material for biomedical applications. Recent discoveries have shown that due to excellent chemical functionality, *Cladophora* cellulosic materials could be easily modified to produce valuable products that are useful for various biomedical applications like immunosorbents, hemodialysis, medicine administration, and viral filtration (Asper et al. 2015; Ferraz et al. 2013; Ferraz and Mihranyan 2014; Metreveli et al. 2014; Wu et al. 2019).

It has been claimed that strategies based on *Cladophora* nanocellulose successfully achieved sustained drug release, which has considerable potential in biomedical applications. Mihranyan et al. demonstrated the effectiveness of *Cladophora* nanocellulose as a sorbent for nicotine. It has been reported that nicotine-containing dosage forms are an effective treatment for minimizing withdrawal effects from nicotine depletion. Even though nicotine can be used in various forms, the free base form is more effective in nicotine replacement therapies and quitting aids. Mihranyan et al. observed that *Cladophora* cellulose powders are suitable nicotine carriers due to the additional advantages of being colorless, tasteless, stable, biocompatible, and somewhat adsorptive. Due to the large surface area and pore volume, the free nicotine was efficiently stabilized on *Cladophora* nanocellulose powder. The study established that nicotine was occupied on open pore surfaces nanocellulose matrices but not in disordered cellulose. High loading capacity, extended-release, and protection from oxidative degradation were accomplished by loading nicotine in porous *Cladophora* cellulose. They found that the crystalline nature of the cellulose powder did not affect nicotine loading or release. However, a large surface area and pore volume could hold more nicotine. They could observe that all nicotine samples were quickly released into water, and the study established

that highly porous *Cladophora* nanocellulose could stabilize and handle liquid drugs (Mihiranyan, Andersson, et al. 2004).

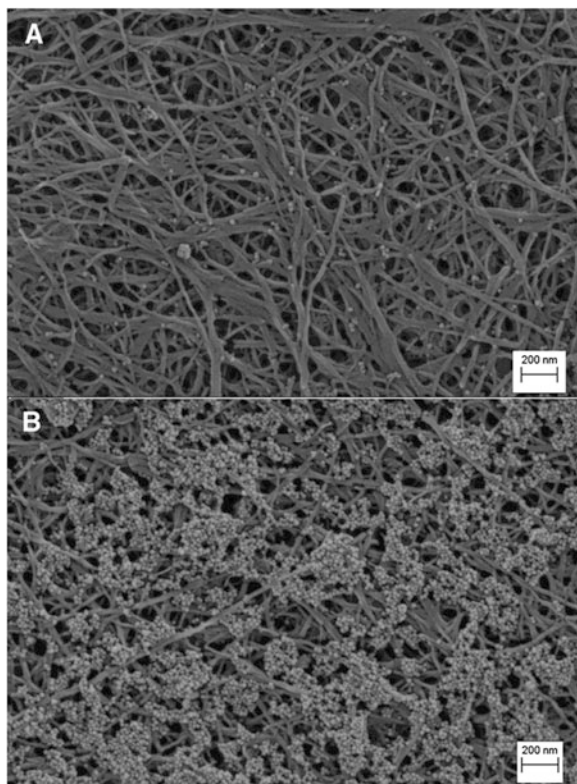
Traditional paper filters have larger pore sizes. Hence cellulose derivatives having smaller pores obtained by the chemical processing of cellulose are used in sterile filtration. However, the functionalization of cellulose is time-consuming since it involves dissolving, derivatizing, and precipitating cellulose, and the final pore size distribution is subject to processing. Besides, most viruses are 20–250 nm, and the filters designed to remove viruses must have a narrow pore size distribution in the 20 nm range. Metreveli et al. examined a *Cladophora* nanocellulose-based membrane filter that can remove virus particles exclusively based on the size-exclusion principle, and their efficacy is comparable to the commercial synthetic and semi-synthetic polymeric counterparts. Their work suggested that using naturally occurring crystalline and porous *Cladophora* nanocellulose-based filters is desirable and economical. The researchers claimed to be the first to show that an unmodified nanofibrous polymer-based membrane could remove virus particles using the size-exclusion principle, producing performance comparable to commercial synthetic polymer-based filters (Metreveli et al. 2014). The cellulose nanofibers used were 30 nm thick and possessed a high level of crystallinity. Comparatively better performance of *Cladophora* nanocellulose-based filter than wood-derived cellulose filters was credited to the better porosity and large specific surface area (about 100 times larger than cellulose made from wood). Using N₂ gas adsorption experiments, they found that *Cladophora* nanocellulose filter paper with an average pore size of 19 nm and a surface area of 88 m²/g can be made by drying wet pulp in a conventional household heat-press. Most of the *Cladophora* nanocellulose membrane pores were smaller than 30 nm. The cellulose membrane had about 35% porosity and was 70 μm thick. Polystyrene latex containing beads of varying sizes (300 nm, 100 nm, 30 nm) were allowed to pass through the cellulose membrane, and SEM images of the filter taken after filtration show that the beads are retained on the membrane surface, demonstrating that the manufactured membrane was capable of filtering particles and viruses via size exclusion strategy. By examining the membrane's ability to retain the 80–100 nm-sized swine flu virus, it was further established that the fabricated *Cladophora* nanocellulose filter could filter out particles of a specific size. According to them, the better performance of the demonstrated filter is because in comparison to other wood-derived nano cellulose, *Cladophora* nanocellulose could keep its large surface area and porosity during conventional drying. After all, their primary nanofibrils are highly crystalline, stiff, and have a large thickness.

Paper filters are fragile when subjected to pressures above a specific threshold, so their industrial application is constrained. In order to use cellulose-based filters at higher pressures, their wet strength must be enhanced, and different methods have been tried to enhance the wet strength of nanocellulose paper. Quellmalz and Mihiranyan developed an entirely nature-derived polymer filter that can remove particles as small as 20 nm, having improved wet strength than pristine *Cladophora* nanocellulose. The wet strength was improved by crosslinking cellulose fibrils with citric acid of the *Cladophora* cellulose filter by crosslinking with citric acid. The

average pore size was 15 nm for the crosslinked *Cladophora* nanocellulose. In order to evaluate the size exclusion filtering potential, tracer retentions tests were carried out with AuNPs having an average size of 20 nm. They performed SEM analysis of the modified and unmodified *Cladophora* cellulose membrane after retention studies, and it was apparent in the SEM images that only a few AuNPs are retained in the unmodified cellulose filter (Fig. 6a), suggesting that majority of tracer particles are escaped through the membrane while, while the surface of the crosslinked nanocellulose paper filter is abundantly covered with Au nanoparticle clusters (Fig. 6b). Therefore they could confirm that crosslinking has drastically improved the filtration capabilities of the cellulose-based paper filter. The crosslinked *Cladophora* nanocellulose membrane could effectively filter Au nanoparticles at a pressure gradient of 37 k Pa without cracking the filter membrane confirming its enhanced wet strength. This team was the first to describe a 100% cellulose-based paper filter capable of removing tracer particles with a particle size of 20 nm, which has tremendous potential for developing a network of nanofiltration devices, such as sterile virus elimination filters (Quellmalz and Mihranyan 2015).

Due to the possibility of retrovirus contamination of therapeutic protein products, the biopharmaceutical industry demands economic and efficient virus removal

Fig. 6 SEM images of the unmodified and cross-linked nanocellulose-based paper filter samples following tracer retention tests (Quellmalz and Mihranyan 2015)



filters, especially for generic therapeutic biologicals. The utilization of cellulose-based filter paper for virus elimination is beneficial due to its cost-effectiveness and ease of preparation. Asper et al. demonstrated that the size-exclusion filter paper made from pure *Cladophora* nanocellulose removes xenotropic murine leukemia virus. Investigations using atomic force microscopic images (Fig. 7a) and pore size calculation based on gas adsorption studies confirmed that the prepared filter paper has an average pore size of 16 nm, which is significantly smaller than the nominal size of xenotropic murine leukemia virus (size range of 100–120 nm). This was further corroborated by SEM studies (Fig. 7b, c). In particular, the ability of the filter paper to hold particles were tested by filtering 100 nm latex beads and 50 nm gold nanoparticles and it was clear from the corresponding SEM images that the latex beads (Fig. 6c) and gold nanoparticles (Fig. 7c) are retained on the surface of the *Cladophora* nanocellulose filter (Asper et al. 2015).

Rocha et al. demonstrated that the hemodialysis potential of the *Cladophora* cellulose could be enhanced by chemical modification of the cellulose nanofibers. Spherical beads composed of sulfonated Cellulose were produced by first oxidizing *Cladophora* nanocellulose to produce DAC, then subjecting the resulting Cellulose to sulfonation with bisulfite (Rocha, Ferraz, et al. 2018). Due to the high surface area, high degree of oxidation, spherical shape, and the ability to tailor the porosity of the sulfonated beads make them exciting candidates for the development of immunosorbent platforms, including their application in extracorporeal blood treatments.

IVIG, which stands for intravenous immunoglobulin, is a valuable plasma-derived biotherapeutic with ample clinical indications in primary and chronic immunodeficiencies and autoimmune disorders, especially neuropathies. Human IVIG and other plasma-derived products must be virus-safe. Viral filtration is used to remove viruses in plasma products. Viral filtering of large amounts of IVIG feed solution can take a long time and be expensive. Therefore, high-capacity filters that can process large volumes of plasma-derived biotherapeutic compounds quickly and cheaply are needed. For the first time, Wu et al. detailed the effectiveness of a virus-removal filter paper made from *Cladophora* nanocellulose in processing human IVIG (Wu et al. 2019).

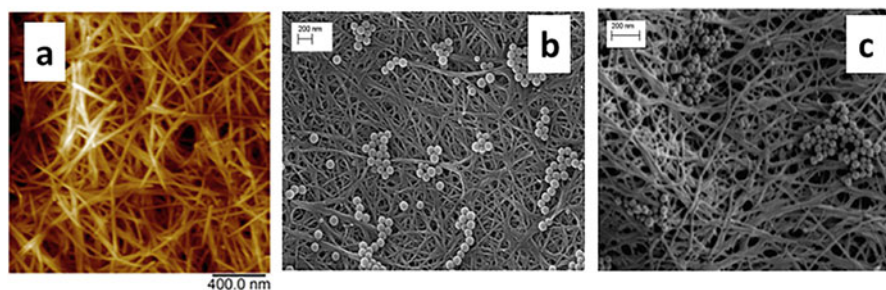


Fig. 7 (a) Atomic force microscopic image of nanocellulose filter paper, (b) SEM images of 100 nm latex beads and (c) 50 nm gold nanoparticle retained over *Cladophora* nanocellulose filter paper (d). (Published with permission (Ref: Asper et al. 2015))

The solute diffusion properties of the materials can be further tuned by incorporating suitable fillers. Ferraz et al. developed an electroactive membrane composed of Cladophora nanocellulose PPy composite for blood purification. They could observe that upon the incorporation of PPy, the average pore size has increased to 53 nm compared to 21 nm of the pure Cellulose. It was observed that the diffusion of tested uremic toxins across the composite membrane was faster since the composite membrane could provide an open structure with a larger pore that facilitates more accessible transport across the membrane (Ferraz et al. 2013).

Due to its high stability, versatility, biocompatibility, adhesive, and therapeutic properties, Cladophora nanocellulose has a wide range of biomedical applications. Hua et al. prepared carboxyl and hydroxy- propyltrimethylammonium functionalized Cladophora nanocellulose derivatives and demonstrated that prepared derivatives are effective as a potential anti-inflammatory agents. *Inflammation* is a localized physical condition when an injury or infection causes the affected body area to swell, turn red, or hurt. The restoration of tissue structure and function, as well as cellular homeostasis, is ultimately caused by the positive host response. Without an inflammatory response, infections, wounds, and tissue damage cannot heal. Cladophora nanocellulose are more effective at blocking inflammation-enhancers like cytokines and inflammation-assisting enzymes than their bulk counterparts because they have a higher surface area-to-volume ratio. Due to their diminutive size, Cladophora nanocellulose may readily pass through most biological barriers, including mucous linings, and even reach the sense organs. The circulatory system receives the Cladophora nanocellulose as soon as they enter the body. The proteins in the blood plasma interact with the nanocellulose inevitably. Despite being less prevalent in blood plasma, serum proteins have a high affinity for binding to Cladophora nanocellulose. Without the use of membrane receptors, based on the location of the Cladophora nanocellulose within the cell, which again depends on its size, different cellular actions are triggered. Some tiny Cladophora nanocellulose are easily endocytosed by most cellular vesicles at greater concentration levels. Neutrophils and macrophages both engage in phagocytosis and macro-pinocytosis. The protein corona that surrounds the nanoparticles makes initial contact with the cell surface receptors when the protein-coated Cladophora nanocellulose interact with macrophages or neutrophils in areas of inflammation and thus reduce the effect (Hua et al. 2015; Patel et al. 2019).

Cladophora nanocellulose-based material is promising for developing affordable and efficient electrochemically controlled ion exchange membranes for batch-wise biomolecule separation demonstrated that Cladophora nanocellulose-PPy composite is used for the electrochemically controlled solid-phase microextraction charged biomolecules like DNA. It is reported that upon oxidation or reduction of electroactive polymers, the ion can be extracted or released when it is kept in contact with the solution. The ions enter or leave the matrix to compensate for the charges during the oxidation/reduction of the conducting polymer matrix. The release and intake of ions depend on the size and nature of the ion. Several research groups have examined the adsorption of DNA on PPy. DNA adsorption on partially oxidized conducting polymers includes diffusion-controlled replenishment of the polymer's counteranions. Some adsorbed DNA can be released by adding additional anions,

albeit at slower rates. This observation suggested that DNA-trapped PPy films can act as cation exchangers for solid phase microextraction (SPME). SPME under electrochemically controlled conditions is more effective than conventional SPME methods since the number and nature of active sites in the electrode membrane and the extraction rate can be readily controlled by adjusting the external current or applied potential.

However, the extraction using pristine conducting polymer alone is not so effective since the outer layer alone is electroactive and can take part in ion extraction. Increasing the polymer layer thickness makes mass transport difficult, further reducing the ion exchange capacity. Razaq et al. found that the ion extraction efficiency can be improved by increasing the surface area of the active PPy layer by coating PPy over high surface area *Cladophora* nanocellulose support. The porous structure of *Cladophora* nanocellulose provided a large surface area for incorporating PPy layers. Electron microscopic studies confirmed that the PPy coating over the Cellulose is only 30–50 nm thick, and the composite could retain the fibrous structure and specific surface area of pure Cellulose. TEM results also revealed that during the extraction, DNA could access the whole volume of the PPy coatings within the composite matrix. The porous structure of the composite and the thin layers of PPy on the high-surface-area cellulose matrix is responsible for the composite's relatively quick adsorption activity. Using the composite material, DNA oligomers of 6, 20, and 40 bases can be extracted, and the amount removed is proportional to the oxidation charge applied in the extraction stage. The conducting paper sheets of PPy-*Cladophora* nanocellulose composite exhibited excellent ion exchange capacities, low internal resistances, and better stabilities. The study demonstrated that the proposed composite material is a good option for batch-wise electrochemically controlled DNA extractions. According to the findings of their research, the developed composite membrane was able to extract all tested DNA oligomers; however, the smallest oligomer was preferentially released during the reduction of the PPy layer, which indicates that the present PPy-cellulose composite may be utilized to separate small DNA oligomers from larger ones (Razaq et al. 2011).

The *Cladophora* cellulose has blood anticoagulant activity which may be due to the inhibition of thrombin, and novel anticoagulants can be derived from *Cladophora* nanocellulose by chemical modification of the surface hydroxyl groups. Scientists have reported that large surface area sulfonated *Cladophora* nanocellulose with a pore diameter ranging from 1 to 100 m could be used instead of heparin as a nontoxic anticoagulant during blood purification (Igor et al. 2016; Majdoub et al. 2009).

Cladophora Nanocellulose as Electrode Material

Cellulose-based paper electrodes have gained much attention compared to traditional metal-based electrodes. Since it is cheap, readily available, biodegradable, flexible, lightweight, and recyclable, the paper makes for a fantastic substrate for electronic devices. To compensate for the poor electrical conductivity of untreated

paper, most paper-based electronics need the deposition or growth of electro-active components onto the cellulose fibers during the manufacturing process. Alternatively, films can be made from electrically conductive materials by assembling components such as carbon nanotubes, graphene, and carbon fibers. Paper-based electronics have exceptional electrochemical performance while retaining considerable flexibility in these approaches. These paper-based electronics achieve significantly high power and energy density because of the cellulosic material's low electrical resistance and compact size (Nyström et al. 2009; Olsson et al. 2012; Wang et al. 2014).

Many applications exploiting the property of conducting polymers to incorporate and exclude ions when they undergo oxidation and reduction have been explored. Using voltammetry and chronoamperometry, several scientists have investigated the relative impact of anion and cation mobility during the oxidation and reduction of conducting polymers. Anions enter oxidized polymers as charge-balancing ions if they are tiny and mobile enough. Since cations migrate to compensate large anions, a high ion absorption capacity is maintained. Charge adjustment by anions and cations is significant in electrochemically regulated solid phase microextraction applications. The ion exchange properties are highly dependent on several factors, including electrochemical properties of the polymer, morphology, pore structure, charge of ions, size of ions, etc. Novel hybrid materials can be developed by combining conducting polymers with high surface area fibers like cellulose, and composites can be used as the economic and environmentally benign alternative for the conventional water softening technique.

Mihiranyan et al. recently established that thin PPy coatings on *Cladophora* cellulose nanofibers are more promising as an ion exchange material. The composite was prepared by incorporating conducting PPy into *Cladophora* cellulose nanofibers by polymerizing pyrrole with ferric chloride oxidatively. The novel conductive paper material, which has a large specific surface area, can be easily molded into paper sheets and bent, twisted, or folded without losing mechanical integrity. It was employed as an ion-exchange membrane and can be immersed in the solution under investigation.

Mihiranyan et al. have demonstrated that by electrochemically switching the polymer between its oxidized and reduced states, these composites can be readily employed for the extraction and desorption of small anions such as chloride ions. The doped composite paper demonstrated excellent chloride ion exchanging capacity (370 °C per g of composite) and cyclic stability when employed as a working electrode in a chloride-containing solution because of the high surface area of the nanofibrous *Cladophora* cellulose and unique electrochemical properties of PPy (Mihiranyan et al. 2008).

Actual water samples generally have a mix of anions and cations, and the presence of another ion also interferes with the ion exchange capacity of the hybrid membrane. Gelin et al. investigated how this PPy-*Cladophora* nanocellulose composite material behaves in water samples containing mixtures of ions since other ions will influence the amount of a specific ion that can be extracted. In order to study the effect of concentration and size of other ions on the ion exchange capacity of a

specific ion, they examined the effects of varying concentrations of nitrate, chloride, and p-toluene sulfonate on the electrochemical characteristics of a novel composite paper material consisting of PPy supported on *Cladophora* cellulose. It can also be seen that the oxidation current was much lower when the experiments were done in an electrolyte containing p-toluene sulfonate instead of electrolytes with smaller anions. This means that the diffusion coefficient of the larger ions is much lower than that of the smaller ions, both in the bulk electrolyte and the composite material. For all three types of electrolytes studied, the current went down as the electrolyte concentration went down. This is because it takes less time for the electrolyte layer next to the composite surface to run out of ions as the electrolyte concentration goes down. It was discovered that nitrate ions are slightly more easily absorbed into the composite than chloride ions. However, the composite's capacity to host major p-toluene sulfonate ions is approximately 50% lower than the smaller ions under the oxidation conditions used in this work. At sufficiently high electrolyte concentrations, most of the composite surface was physisorbed by the anions before they diffused into the bulk of the polymer, and the distance between surface sites roughly matched the sizes of the anions. In a solution containing both p-toluene sulfonate ions and chloride ions, it was revealed that high concentrations of the more significant p-toluene sulfonate anion might inhibit the transit of chloride ions into the composite, whereas low quantities may promote the transport. Even for large anions, the present results demonstrate that the combination of the thin polymer covering and the large specific surface area of the composite results in a high ion absorption capacity (Gelin et al. 2009).

Razaq et al. prepared chopped carbon filaments that reinforced PPy–*Cladophora* cellulose composites and symmetrical supercapacitor systems containing paper-based composite electrodes with high charge capacities owing to the fabrication of lower resistance. The excellent cyclic stability exhibited by these electrodes demonstrated inexpensive and ecologically acceptable paper-based energy storage devices (Razaq et al. 2012).

Tan et al. prepared *Cladophora* cellulose coated with PANI nanocomposite to create a paper-based electrode for lead detection. Polymerization was done in the dispersion of threads of *Cladophora* cellulose, which resulted in the formation of thin layers of PANI over cellulose while keeping the highly crystalline structure of cellulose intact. The composite was formed into a working electrode, and the cyclic voltammetry (CV) profile displayed characteristic peaks of PANI, and the peak current was significantly higher than the corresponding cellulose electrode. PANI-cellulose modified electrodes exhibited quasi reversible redox processes and good cyclic stability. The PANI-cellulose composite was used to fabricate a low-cost electrochemical sensor for lead ions, and sensing parameters were determined employing stripping voltammetric techniques. It was reported that the developed sensor could show a linear working range of 0.2–1.0 ppm and a low detection limit of 0.0721 ppm for Pb^{2+} . This work high lightened the potential of cellulose-based electrochemical sensors for rapid water pollution monitoring since there was no significant difference in the quantity of lead from that estimated by conventional atomic absorption spectroscopy techniques (Tan et al. 2018).

Wang et al. also prepared a conductive composite of Cladophora cellulose with PPy by chemical polymerization of pyrrole in the presence of dispersed cellulose nanofibers, and the resulting composite was employed as binder-free electrodes. The prepared composite was compressed by applying external pressure, and it was observed that the density and porosity of the electrode material thus obtained were influenced by the pressure applied. As the applied pressure increased, the electrodes became thinner and denser. Electron micrographs indicated that the compressed sample had a more compact structure and flat surface. The uniform coating of PPy on Cladophora cellulose resulted in uniform interweaved PPy@nanocellulose fibers with a 90 nm average diameter. Entangled nanofibers form voids with tens-of-nanometer pores. For the sample exposed to higher compression pressure, the material displayed small voids that suggest the material remained porous, and the interconnected pore structure was well preserved throughout the material. N₂ sorption studies revealed that mesoporous microstructures were retained in the uncompressed and compressed samples, while specific surface area measurements suggested that compression has reduced the effective surface area. They could observe that the electrochemical performance of the nanocellulose-PPy was not hindered by the reduction in the surface area since energy storage occurs mainly through oxidation and reduction of the PPy layer rather than charging the double layer. It is suggested that cellulose-incorporated porous material could allow the PPy layer to rapidly oxidize and reduce throughout the material and enhance energy storage. Their findings suggested that the compacted materials have well-maintained mesoporous structures, and the PPy layer on the cellulose fibers should give rise to a suitable surface wettability concerning the aqueous electrolyte, suggesting that these compact materials should serve as all polymer-based electrodes with higher energy densities than their new counterparts. Remarkably, they could find that when the electrodes were compressed, it was discovered that their volumetric capacitance increased, but their gravimetric capacitance decreased. The compact composite sample has far higher volumetric capacitance than compact porous carbon. It has been found that prepared electrodes had significantly higher areal capacitances than carbon and polymer-based electrodes, reaching values as high as 5.66 F cm⁻². This is a significant finding since it suggests that the energy density of electrodes can be enhanced simply by compressing them, whereas earlier efforts to reduce porosity tend to lower capacities due to changes in mesopore distribution. While pressing in water, the polar environment surrounding the fiber screens the surface charges, resulting in an altered fiber alignment (Wang et al. 2014).

Due to better crystallinity, high thermal stability, and ease of manufacture, Cladophora cellulose is considered an excellent candidate as an alternative for conventional polyolefin separators in lithium-ion batteries. Pan et al. have demonstrated that the renewable and inexpensive Cladophora cellulose is highly crystalline and porous, so it is suggested to be easier for lithium ions and electrolytes to move through Cladophora cellulose-based separators. Due to high wettability, Cladophora cellulose separators are anticipated to rapidly absorb electrolytes generally used in lithium-ion batteries, hence facilitating the electrolyte filling process. They could observe that Cladophora nanocellulose was stable without any visible deformation

even at temperatures as high as 150 °C; it could be considered a safer choice, especially for high power/temperature battery applications. The lithium-ion batteries with *Cladophora* cellulose separators with good cyclic stability were obtained with 99.5% capacity retention after 50 cycles (Pan et al. 2016).

Recent studies reported that silicon-based anodes are appropriate for lithium-ion batteries due to their cost-effectiveness and high theoretical specific capacity. However, silicon undergoes enormous volume changes during charging/discharging, causing the anode to break up and lose contact with the current collector, causing the battery's capacity to drop quickly. It is reported that such issues can be resolved by incorporating polymers, nitrocellulose, or carbon nanomaterials with silicon. Wang et al. demonstrated that lightweight, flexible *Cladophora* nanocellulose-based silicon paper electrodes could be made without post-treatment. Due to the silicon nanoparticles' uniform distribution and strong adhesion to the 3D conductive flexible CNT-*Cladophora* nanocellulose cellulose network, these electrodes exhibit high areal capacities (2.5 mAh cm⁻²) and specific capacities (3200 mAh g⁻¹). The better electrochemical performance and industrially scalable preparation method are promising for developing lightweight, cost-effective, high-energy anodes for lithium-ion batteries (Wang et al. 2015).

Olsson and colleagues explored the root cause of self-discharge in positively charged PPy. Consequently, they assessed the potential loss under open circuit conditions in a three-electrode configuration with PPy/cellulose composites as the working electrode. *Cladophora* nanocellulose-PPy composites were fabricated by combining pyrrole solution (in 0.5 M HCl) and FeCl₃ solution with *Cladophora* nanocellulose dispersion. The self-discharge of PPy was measured by charging the composite with a potential step to 0.53 V for 600 s, followed by monitoring the open circuit potential decay. They hypothesized that the self-discharge of the positive PPy electrode is caused by an activation-controlled Faradaic reaction that results in the partial degradation of the polymer. They proposed that self-discharge in PPy is caused by a range of degradation products, including maleimide, derived from a common intermediate generated in a polymer-intrinsic and rate-limiting Faradaic process. Due to the exponential dependency of the self-discharge rate, the primary source of self-discharge becomes insignificant at potentials below 0.25 V vs Ag/AgCl; hence, PPy composites can be operated under stable conditions below this potential limit in applications involving electrical energy storage (Olsson et al. 2014).

Tammela and his co-workers investigated the effect of cell design on cell resistance in symmetric PPy and cellulose-based electric energy storage devices utilizing chronopotentiometry and ac impedance measurements with various separator and electrode thicknesses. With SEM micrographs of PPy/cellulose and PPy/cellulose/carbon chopped fiber composites, the morphologies of the composites were analyzed. Both samples displayed the characteristic fine fibrillar structure of PPy-coated *Cladophora* cellulose samples. Based on constant current and ac impedance measurements, it has been demonstrated that the cell resistance of symmetric PPy/cellulose composite-based energy storage devices is primarily governed by the resistance of the current collectors and the contact resistances between the electrodes and the current collectors. They discovered that the resistance of the

PPy and cellulose composite electrodes was insignificant. However, the resistance of the separator soaked in electrolyte increased linearly as the thickness of the separator increased. In the absence of sufficient external pressure on the cell, it was determined that the employment of platinum foils (with negligible resistance) as current collectors led to significantly higher contact resistances than graphite foils. Platinum current collectors are not feasible alternatives to graphite foils for enhancing the performance of energy storage devices due to their high cost. Also, the incorporation of chopped carbon fibers into the electrode material and the application of external pressure across the cells were found to reduce the charge transfer resistance as determined by ac impedance measurements due to decreased contact resistances between the electrodes and the current graphite collectors. It is also evident from their study that the separator should be as thin as feasible in order to reduce cell resistance and that the porosity of the electrodes guarantees that sufficient electrolyte is maintained within the cell. These findings enable the creation of energy storage devices based on the current type of affordable PPy and cellulose composite electrodes (Tammela et al. 2014).

Etman et al. demonstrated that electrode materials having high charge/discharge rates for flexible charge storage devices could be derived from *Cladophora* cellulose by incorporating electroactive fillers. The ternary composite of nanostructured molybdenum oxide (MoO_{3-x}), carbon nanotube, and *Cladophora* cellulose obtained via a one-step route have resulted in a uniform distribution of molybdenum oxide within the *Cladophora* cellulose-carbon nanotube (CNT) matrix confirmed by the electron microscopic and energy dispersive X-ray spectroscopic studies. The MoO_{3-x} -CNTs-*Cladophora* cellulose-based electrode delivered a reversible capacity of 30 Cg^{-1} at current densities between 0.78 and 78 Ag^{-1} . The observed low capacity suggested that only the surface layer of the oxide was electroactive. Also, electrodes can be cycled for more than 30,000 cycles without a significant reduction in capacity loss and severe structural change in the active electrode material. Based on the peak current and potential redox analysis, it is established that the electrode kinetics were fast and that the polarization was caused primarily by the iR fall. The developed MoO_{3-x} -CNTs-*Cladophora* cellulose electrodes demonstrated excellent cyclability and rate capability, ascribed to the redox reaction's surface-confined nature, the absence of diffusion limitations, the electrodes' high electrical conductivity and flexibility, and the reasonably moderate rise in iR drop (Etman et al. 2019).

Keskinen et al. prepared symmetric and asymmetric supercapacitors using either two PPy composite electrodes, one PPy composite, or one activated carbon electrode, respectively. The fabricated PPy composite electrodes utilized *Cladophora* nanocellulose as the substrate or binder. The electrochemical characteristics of the symmetric and asymmetric supercapacitors containing cellulose, PPy composite electrodes, and activated carbon were investigated. Capacitance is better for symmetric composite-based devices over asymmetric supercapacitors, while an asymmetric supercapacitor combining PPy and activated carbon electrodes seems to have a longer lifetime. The specific capacitance of PPy electrodes was roughly 200 F g^{-1} depending on the manufacturing method, generating supercapacitors with capacitances between 0.45 and 3.8 F and above 90% energy efficiency. An asymmetric

device with activated carbon positive and PPy negative electrodes lost 5% capacitance after 14,300 cycles, whereas the PPy sheet component's efficiency did not change. By using *Cladophora* nanocellulose, the study showed that the printing techniques they demonstrated could be used to make many supercapacitors that are cheap and safe for the environment (Keskinen et al. 2015).

Liu et al. fabricated a novel binder-free nitrogen-doped carbon paper electrode derived from a nitrogen-rich PPy/cellulose-chopped carbon filaments (CCFs) composite for Li-O₂ batteries. The preparation of electrode material is economical and environmentally friendly as the composite is obtained by in situ polymerization of pyrrole in *Cladophora* cellulose. The composite of nitrogen-rich PPy and cellulose-CCFs had a bird's nest microstructure capable of providing the free-standing electrode with mechanical durability, rapid O₂ diffusion, and sufficient space for discharge product deposition. N-doping could promote electrochemical reactions. The discharge capacity of 8040 mAh.g⁻¹ was observed for a nitrogen-doped carbon paper electrode Li-O₂ cell, along with satisfactory cyclic stability and good coulombic efficiency (81% at the current density of 0.2 mA cm⁻²). These studies established that by using *Cladophora* nanocellulose as the support matrix for incorporating active fillers, novel non-metallic, binder-free electrode materials have improved energy density for the Li-O₂ batteries can be developed (Liu et al. 2016).

Using PPy-coated *Cladophora* nanocellulose composites and highly porous carbon nanofibers-based materials that can be easily obtained from the PPy-cellulose composite itself, Tammela et al. demonstrated that all-organic material-based asymmetric energy storage devices could be manufactured. The PPy-cellulose was subjected to a heat treatment in the presence of nitrogen, which resulted in the production of the porous carbon nanofiber-based material. Studies using electron microscopy and x-ray photoelectron spectroscopy demonstrated that the heat treatment results in forming a porous carbon nanofiber material were almost topologically identical to the PPy-cellulose composite used originally. Additionally, it was shown that the electrochemical performances of PPy-cellulose and carbon nanofiber materials varied greatly from one another and that the combination of these materials in asymmetric supercapacitor cells is favorable. Asymmetric supercapacitor performance was evaluated using galvanostatic charge, discharge, and electrochemical impedance spectroscopy. With a current density of 156 mA/cm², the asymmetric devices had a capacitance of 11 F/g, and the capacity dropped by about 23% after 1000 cycles with a current density of 20 mA/cm². The supercapacitor could undergo galvanostatic charge and discharge for 1000 cycles at 20 mA/cm² with acceptable stability in 0.2% NaCl. This work is a massive step toward all-organic, renewable energy storage devices with commercially viable mass loadings, capacitances, and energy densities (Tammela et al. 2015).

Nystrom et al. produced a robust, lightweight, flexible PPy-cellulose composite that can be molded and rolled to construct miniature energy storage devices and paper-based batteries. Cost-effective, recyclable paper batteries require abundant cellulose and simple composite construction. The composite material was created readily by oxidizing pyrrole on *Cladophora* cellulose with Fe(III) chloride. The method they describe has limited cell potential compared to Li-ion batteries. However, it offers

significant promise for applications where Li-ion batteries are difficult to employ, such as affordable large-scale or flexible energy storage devices integrated into textiles or packaging materials. Cladophora nanocellulose composite conductive paper material has a specific surface area of $80 \text{ m}^2 \text{ g}^{-1}$, and its batteries lose only 6% of their capacity over 100 charge and discharge cycles. The cellulose and PPy-based batteries have charge capacities between 25 and 33 mAh g^{-1} or 38–50 mAh g^{-1} , opening new opportunities for environmentally friendly, cost-efficient, up-scalable, and lightweight energy storage systems (Nyström et al. 2009). This paper-based battery technology is compatible with fast charging rates and has good cycling stability, making it ideal for future high-performance energy storage systems.

Cladophora Cellulose as Eco-Friendly Packaging Material

The primary contributor to plastic pollution in the environment is using plastic for packaging. Therefore, it is desirable to employ readily available, biodegradable cellulose as an environmentally friendly packaging material. Despite its excellent tensile strength and water resistance, cellulose is difficult to process due to its fragility. The processability can be improved by introducing materials like starch which is also environmentally friendly. Steve and his coworkers studied the usefulness of Cladophora nanocellulose-starch-based bio-composite as an alternative eco-friendly packaging. Starch has poor mechanical strength and water-resistant properties, but blending starch with Cladophora cellulose which has excellent mechanical strength and good water-resistant properties has resulted in the composite material having desired properties. The Cladophora cellulose microfibrils are much broader than that derived from wood, so the Cladophora cellulose has superior crystallinity.

Additionally, the distinctive web-like structure of Cladophora cellulose resulted in improved mechanical strength. Steve et al. made bio-composite film from cellulose and starch by adding glycerol as a plasticizer to improve processability and flexibility. They found that the developed bio-composite film had greater tensile strength and elongation than the new components – the bio-composite film comprised 70% starch and achieved a tensile strength of 80.16 MPa. The study revealed that the water resistance properties of the starch film were improved during the composite formation since cellulose inhibits interactions between starch and water. The bio-composite films exhibited superior biodegradability than new cellulose film. According to the results of the study, it is established that Cladophora cellulose has the potential to be used as a green packaging material that is both eco-friendly and long-lasting (Steven et al. 2020; Sucaldito and Camacho 2017).

Conclusion

This chapter presents a comprehensive discussion on the extraction, unique characteristics, and applications of the Cladophora-derived nanocrystalline cellulose. It is demonstrated that the unique properties like tunable porosity, larger surface area,

biocompatibility, and rich surface functionality could find excellent applications in exciting fields like hemodialysis, virus filtration, environment pollution remediation, energy storage, and electronics. Notably, utilizing *Cladophora* biomass to extract cellulose offers a remedy for biowaste accumulation and is economically viable. The review revealed that intensive research is needed in academia and industry to develop innovative extraction processes that would result in efficient processes for getting high-quality cellulose in good yield. This research was required in order to develop novel industrial extraction processes. To improve the properties of nanocellulose and expand the application sphere of nanocellulose, especially in medical and industrial technologies, additional work must be done to modify the current material. We anticipate that the structure, biochemistry, and electrochemistry of *Cladophora* nanocellulose, detailed here, can further enhance and expand the exploration, application, and future development of various cellulosic materials from numerous marine and agricultural biomass.

Cross-References

- Applications of Biopolymer-Based Nanofibers and Nanomembranes
- Biopolymer-Based Composites
- Biopolymer-Based Nanocomposites
- Biopolymer-Based Nanocomposites for the Treatment of Organic Pollutants from the Synthetic Wastewater
- Biopolymers
- Nanocellulose-Based (Bio)composites for Optoelectronic Applications
- Nanofibers and Nanomembranes of Biopolymers
- Optical Properties of Biopolymers
- Piezoelectric Properties of Biopolymers

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Cellulose Nanocrystals (CNCs) Supported Inorganic Nanomaterials for Catalytic Applications

32

M. H. Rubiya, Krishnakumar Melethil, Albin James, Sharon Varghese, and Bejoy Thomas

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M. H. Rubiya

Mahatma Gandhi University Centre for Research in Chemistry, Mar Athanasius College,
Kothamangalam, Kerala, India

K. Melethil · S. Varghese · B. Thomas (✉)

Department of Chemistry, Newman College, Thodupuzha, Kerala, India

e-mail: bejoy.thomas@newmancollege.ac.in

A. James

Mahatma Gandhi University Centre for Research in Chemistry, Newman College, Thodupuzha,
Kerala, India

Mahatma Gandhi Centre for Teacher Education, Thodupuzha, Kerala, India

Abstract

The nanoscale version of cellulose, known as nanocellulose (NC), has emerged as a promising green material thanks to its distinct properties, including its renewability, biodegradability, ecologically benign nature, and abundant natural occurrence. Many of the beneficial qualities of cellulose are also present in NC, such as their low density, nontoxicity, biodegradability, thermal stability, mechanical properties, reinforcing capabilities, and tunable self-assembly in aqueous conditions. The surface of NC is one of their most interesting features since it may be altered in a wide variety of ways by adding functionalizing chemicals. Esterification, etherification, amidation, and urethanization (all hydrophobic decorations), phosphorylation, sulfonation, TEMPO-oxidation, and carboxymethylation (all hydrophilic surface modifications) are just some of the ways documented for surface functionalization of NC. It is possible to create NC/inorganic composite materials by functionalizing the NC surface with inorganic metal ions, complexes, or compounds. The catalytic transformation of organic molecules is one area where such inorganically modified NC finds use. An in-depth analysis of how cellulose nanocrystals (CNCs) can be inorganically functionalized for use in catalysis has been done. Using CNCs to facilitate the catalytic transformation of organic compounds through oxidation, reduction, coupling, and electrocatalytic reactions has environmental benefits.

Keywords

Nanocellulose · Inorganic functionalization · Catalytic applications · Reduction and oxidation reactions · Coupling reactions · Electrocatalysis · Photocatalysis · Environmental remediation

Introduction

Since the dawn of civilization, humans have used various forms of written and visual communication to convey meaning and information to one another. In the past, humans have used everything from rocks and wooden pieces to leaves to write down their thoughts and pass on their knowledge to future generations. These days, most people use wood pulp paper since it is both affordable and widely available. The main chemical components of wood pulp are cellulose, lignin, and pectin. Scientific advances have allowed researchers to isolate or synthesize cellulose from a wide variety of sources, including wood, leaves, algae, fungi, bacteria, fibers, grasses, husk, and marine animals. Payen discovered cellulose's fibrillar arrangement, which makes a significant contribution to plants' mechanical strength. It is a staple of plant life and one of the most common organic substances. Each year, between 1010 and 1011 metric tons of cellulose biopolymer are produced. Cellulose polymers are formed when D-glucopyranose (glucose) molecules are bonded to one another via 1,4-C–O–C-glucosidic linkages. Cellulose molecules have the chemical formula (C₆H₁₀O₅)_n, where n is the number of repeating sugar units, and their degree of

polymerization (DP) has a major impact on the properties of cellulose-containing materials. The cellulose repeating unit, known as anhydrocellobiose, consists of two glucose molecules. The DP of native cellulose can be anywhere from 1500 to 3500, depending on its source and degree of processing. Cellulose, hemicellulose, lignin (Fig. 1), waxes, etc., form a traditional hierarchical structure in plants that gives them their strength and other special features. Cellulose, a biopolymer made from the α -D-glucose monomer, is the primary component of plants that provides structural support and reinforcement along with hemicellulose and lignin. It has been identified as the most abundant biopolymer with specific properties such as hydrophilicity, hydrophobicity [and thus a typical hydrophilic to lyophilic balance (HLB)], biodegradability, nontoxicity, good mechanical strength, etc. Nanocrystalline particles, which are renewable, biodegradable, and nontoxic, can be synthesized using cellulose because of the material's semi-crystalline structure. Isolating the nanoparticles of this substance from the biomass requires a top-down mechanically or chemically induced deconstruction technique (Dufresne 2013).

The wide spectrum of possible uses and low manufacturing cost of cellulose make it a material of particular interest for its positive impact on the environment. Due to its singular form, size, surface chemistry, and high degree of crystallinity, it possesses great mechanical strength, reinforcing capabilities, and tunable self-assembly in aqueous mediums. Cellulose nanocrystals (CNC), cellulose nanofibers (CNF), and bacterial nanocellulose are the three most common types of nanocellulose used in nanotechnology due to their distinct origins (or more precisely, due to the extraction protocol or synthetic approaches), morphology, particle size/shape, crystallinity, etc. (BNC). It is possible to extract CNCs and CNFs using either direct chemical procedures or a mix of chemical and mechanical channels, such as enzyme-chemical or mechano-enzymatic hydrolysis approaches, whereas BNC is obtained through bio-formation utilizing

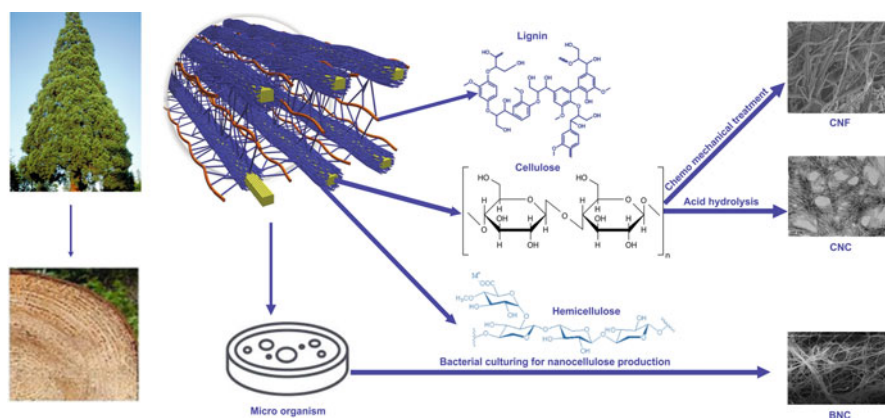


Fig. 1 A diagram demonstrating the hierarchical structure of nanocellulose from the top down. (Permission granted to reproduce BNC and CNC photos from Fan Li et al. *Holzforschung* doi:10.1515/hf-2018-0251 and Nirmal N et al. *RSC Adv.* DOI: 10.1039/D0RA04649C)

bacterial cultures. These are utilized in many different industries, including those dealing with food, textiles, autos, study aids, kitchen aids, polymers, paints, chemical manufacturing, fuel generation, etc. Modifications to the surface of nanocellulose produced from waste biomass or bacterial cultures activate the catalyst's broad activity in organic transformations and biofuel production. In this review, we discussed about the structure, modification of cellulose using metal based catalysts and its applications, especially towards diverse types of organic transformations.

Catalysis is employed in roughly 80% of all manufacturing processes, including the production of anything from sulfuric acid to acetone to phenol, etc. These three considerations highlight the critical nature of this physical process. The production of chemicals has become more sustainable, which has three benefits: (1) it protects the environment, (2) it helps produce things that people need in the twenty-first century, and (3) it aids the world's major economies in their quest for growth in a sustainable way. Homogenous catalysts, heterogeneous (solid) catalysts, heterogenized homogeneous catalysts, and biocatalysts are the four primary types of catalysts. It is easier to handle, store, recycle, and separate the catalyst from the product, among other benefits, when using a heterogeneous catalyst as opposed to a homogeneous or heterogenized homogeneous catalyst, which is an important consideration when designing eco-friendly technologies. Several materials are used as commercial catalysts, including vanadium oxides, iron and manganese oxides, platinum and rhodium gauze, zeolites, clays, alumina, zirconia, and others. Exploring nanocellulose's potential as a catalyst support for various reactions has witnessed a spike in research in recent decades. Nanocelluloses, or nanomaterials based on cellulose, are currently the focus of extensive research. Thanks to its enhanced chemical, colloidal, optical, and mechanical capabilities, bacterial nanocellulose has been selected as a promising catalytic support among the numerous nanocelluloses (CNC, CNF, and BNC). So, although these sustainable heterogeneous catalysts have not yet been deployed in a commercial setting, a lot of work has gone into their creation and usage.

This chapter details the state-of-the-art extraction techniques used to create metal/CNC nanocomposites with excellent metal dispersion, high surface area, and high catalytic activity. To enhance its physicochemical properties and aid in efficient metal stabilization, synthetic procedures were devised to modify the morphological/chemical functionality of CNC. This resulted in enhanced catalytic activity. The organic reactions that can be catalyzed by catalysts based on CNC are then illustrated graphically. A perspective for the future of the discipline is provided, as are developments in the study of materials based on CNCs for (nano)catalysis that are expected to occur in the near future.

Hierarchical Structure and Source of Cellulose

The composition of lignocellulose biomass varies depending on its source, but it always includes the three structural components cellulose, hemicellulose, and lignin (Fig. 1). Lignin and hemicellulose form a matrix around the cellulose fibrils in a lignocellulosic composite. Hydrogen bonds hold cellulose to hemicellulose within

the biocomposite system, and covalent bonds hold it to the lignin framework outside of the biocomposite. Hemicellulose is assembled both inside of the cellulose and between the cellulose and lignin (Klemm et al. 2005; Moon et al. 2011; Thomas et al. 2018).

Significant portions of the plant cell wall are made up of hemicellulose group of materials. Hemicelluloses are heteropolysaccharides with a diverse range of chemical compositions, including pentoses (xylose and arabinose), hexoses (glucose, mannose, galactose, and fructose), and sugar acids (glucuronic acid and galacturonic acid). Although having a lower DP value than cellulose, hemicellulose is highly chemically soluble, making its extraction from lignocellulosic biomass a simple and straightforward process (Klemm et al. 2005; Moon et al. 2011; Thomas et al. 2018). The physical properties of hemicelluloses contribute to make up the bulk of the fiber; they are white solid solids that are rarely crystalline or fibrous in character. By interacting with cellulose and, in some walls, with lignin as a lignocellulosic biomass composite, hemicelluloses play their most essential biological role as a component of the cell wall (Fig. 1).

Lignin, a phenolic (phenolics – having a -OH group attached to an aromatic ring) polymer, is found in all land plants and possibly some marine ones as well (Fig. 1). To sum up, they are plant polymers made up of phenylpropanoid building blocks and accounting for the vast bulk of wood's methoxyl content. Lignin is made up of a collection of irregular propyl phenol units, some of which are coniferyl alcohol, sinapyl alcohol, and p-coumaryl alcohol. Ether, ester, and carbon-carbon bonds are used to bring these subunits together. Plants get their tensile strength from lignin because it fills the voids between cellulose, hemicellulose, and other cell components. Lignin is a natural substance found in plants that improves the tensile and compressive strength of the cell wall (Klemm et al. 2005; Moon et al. 2011). It is believed that it helps terrestrial plants evolve by making them more resistant to the weight of the Earth's atmosphere. Water can more easily travel up the xylem tissues of a plant thanks to the lignin's waterproofing qualities. Lignin's carbolic composition gives it powerful antifungal properties, and it is often rapidly deposited in response to fungi attack or other injury, shielding the plant from potentially fatal infections.

Cellulose is a linear-chain polymer with a greater number of hydroxyl groups in the thermodynamically preferred C4 and C1 conformation (Klemm et al. 2005). It is a carbohydrate polymer composed of repeating -D-glucopyranose molecules covalently linked through acetal functions between the equatorial OH group of C4 and the C1 carbon atom (Fig. 2). With each consecutive glucose residue rotated over an angle of 180 degrees about the molecular axis, the anhydroglucose units adopt a chair configuration (Eichhorn et al. 2001). Cellulose's strong axial stiffness and linear structure come from the intrachain hydrogen bonding between the hydroxyl groups and oxygens of the neighboring ring molecules. This, together with the action of Van der Waals forces, promotes the parallel stacking of numerous cellulose chains, which results in elementary fibrils that eventually aggregate into bigger microfibrils (Moon et al. 2011).

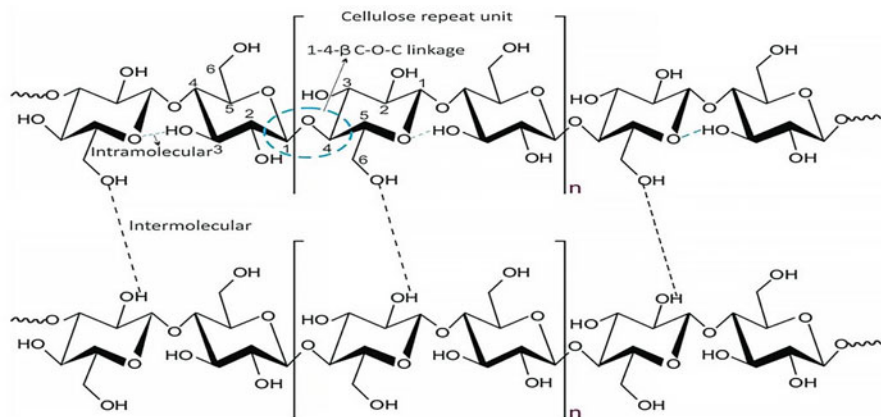


Fig. 2 Cellulose, shown in simplified form, is a linear polymer composed of β -D-glucopyranose units that are covalently bonded by (C1–C4) C–O–C glycosidic bonds and characterized by intramolecular hydrogen bonding between units as well as intermolecular H-bonding between chains

Cellulose's numerous crystalline forms, or polymorphs, result from subtle shifts in the packing and molecular orientation of its chains as a result of the complicated intra- and intermolecular hydrogen-bonding (Fig. 2) arrangements. According to the literature (Nishiyama et al. 2003; Nishiyama et al. 2002; O'sullivan 1997), cellulose can be found in six distinct polymorphs: cellulose I, II, III, IIII, IVI, and IVII. Crystalline cellulose I is composed of two sub-polymorphs: cellulose I, which is found mostly in algal and bacterial cellulose, and cellulose I, which is found primarily in the cell wall of higher plants (Imai et al. 1999). The percentages of cellulose I and cellulose I within the cellulose are different depending on the source of the cellulose; cellulose I contains a cellulose chain in a triclinic unit cell, whereas cellulose I unit cells contain two chains with a monoclinic structure. Cellulose II, produced through regeneration or mercerization, has a monoclinic structure and an antiparallel configuration of chains grouped in a 3D hydrogen-bonded network, rendering it more thermodynamically stable than cellulose I. Cellulose's crystalline structure can be altered into cellulose III by treating it with amine-containing substances like ammonia or ethylenediamine; cellulose III has two sub-polymorphs, IIII and IIII, which are distinguished by the orientation of their cellulose chains (Wada et al. 2004). Cellulose III's crystalline structure changes into cellulose IV (Zeronian and Ryu 1987), which has two sub-polymorphs, IVI and IVII, when heated in water, glycerol, or formamide to temperatures up to 180 °C.

Plants benefit from the structural support, reinforcement, and other special features provided by cellulose, which has β -D-glucose as its monomer, hemicellulose, and lignin. By synthesizing nanocrystalline particles, this most abundant biopolymer can be put to use since its features, such as hydrophilicity, hydrophobicity, biodegradability, non-toxicity, good mechanical strength, etc., can be exploited. As a result, research into the synthesis of nanocrystalline cellulose, its inorganic functionalization, and applications in

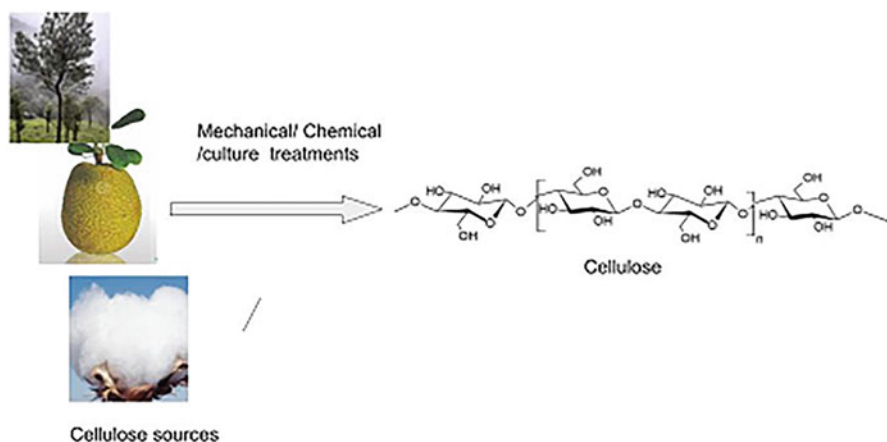


Fig. 3 Schematic representation of the extraction of cellulose from different plant sources

the field of catalysis, with a focus on using renewable resources rather than petroleum-based sources, is now a hot topic. A linear homopolymer of glucose (Pacsu 1947), insoluble in water, cellulose with a β -1,4-C-O-C glycosidic linkage consists of crystalline and semi-crystalline or amorphous areas connected by inter- and intramolecular hydrogen bonding. Hydrogen bonds unite cellulose and hemicellulose in lignocellulosic biomass, and lignin is covalently bound to cellulose (Li et al. 2019) (Fig. 2). Xylans, a uronic acid, and arabinose are all examples of the class of complex carbohydrates known as hemicellulose. Lignin is a naturally occurring, irregular phenolic polymer made up of propyl phenol units such as coniferyl alcohol and sinapyl alcohol, as well as a small amount of p-coumaryl alcohol, which are cross-linked using carbon-carbon, ester, and ether links.

Cellulose can be extracted from a wide variety of plant and animal sources, including algae, fungi, bacteria, invertebrates, and amoeba (protozoa), and every plant and animal from the lowest to the highest trophic levels (Habibi et al. 2010). Cellulose can be made both from the ground up (from trees, cotton, and crop waste) and from the top down (through bacterial fermentation of glucose) (Trache et al. 2016) (Fig. 3).

Nomenclature and Types of Nanocellulose

In the 1950s, Ranby was the first to discover that colloidal suspensions of cellulose could be generated through the controlled sulfuric acid-catalyzed breakdown of cellulose fibers (Habibi et al. 2010). Nanocellulose refers to materials whose lateral dimensions fall between 5 nm and 20 nm and whose lengths range from a few tens of nanometers to several microns. Renewal, hydrophilicity, high mechanical qualities, biocompatibility, broad chemical-modification capacity, and the generation of various semicrystalline fiber morphologies were augmented with characteristics,

including transparency, electrical conductivity, strength, and lightweight (Klemm et al. 2011).

Cellulose nanocrystals (CNC), cellulose nanofibers (CNF), and bacterial nanocellulose are the three primary categories of nanocellulose (Fig. 4), each with its own unique origin, morphology, particle size/shape, crystallinity, etc. (BNC). Highly crystalline cellulose nanocrystals are obtained using a multistep chemical process that includes alkali treatment, bleaching, and acid hydrolysis to eliminate the amorphous portions of lignocellulosic material (Gupta and Shukla 2020). Cellulose nanofibers are produced by mechanically disintegrating cellulose or by subjecting it to controlled acid hydrolysis, both of which result in the breaking of the long, thick fibers. Bacterial nanocellulose, also known as high-purity cellulose, can be synthesized by microorganisms such as *Gluconacetobacter* bacterial strains such as *G. xylinus* in aqueous culture conditions in a matter of days to weeks (Abol-Fotouh et al. 2020).

Cellulose nanocrystals (CNCs) are rod-like cellulose crystals that are formed by the removal of amorphous parts of cellulose. Their widths and lengths range from 5 to 70 nanometers and 100 nanometers to several micrometers, respectively. Cellulose nanocrystals are also known as CNCs. The crystallinity of cellulose can range from 54 to 88% depending on its origin and isolation technique (Moon et al. 2011). For the purpose of CNC extraction, many different types of biomass were used, including wood, cotton, and agricultural wastes like wheat straws or rice, garlic, tomato, kenaf, coir, sugarcane bagasse, and algae (Thomas et al. 2018). Traditional chemical methods for isolating cellulose nanocrystals used pretreatments

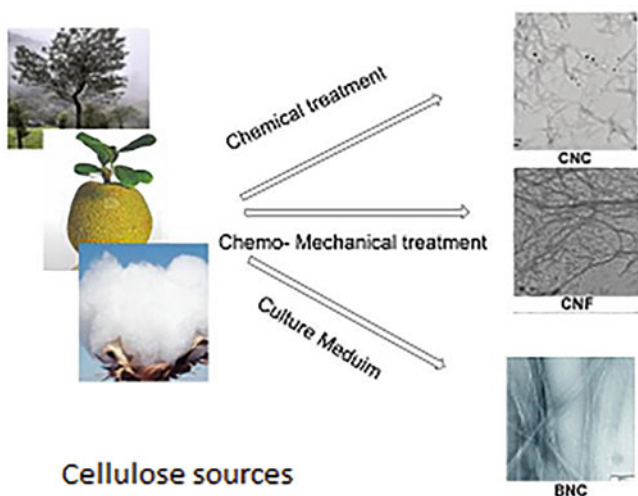


Fig. 4 The hierarchical structure of nanocellulose from the top down, together with the many forms of cellulose, depicted schematically. (Permission granted to reprint BNC and CNC photos from Fan Li et al. *Holzforschung* doi:10.1515/hf-2018-0251 and Nirmal N et al. *RSC Adv.* DOI: 10.1039/D0RA04649C)

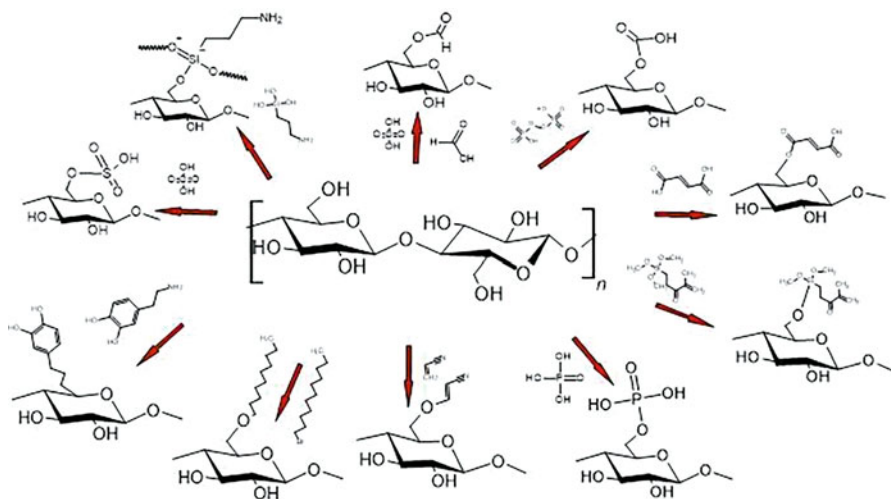
with alkali and bleaching, followed by acid hydrolysis of lignocellulosic biomass. The pretreatment phase involves the elimination of non-cellulosic substances such as waxes, lignin, pectin, and hemicellulose. To get rid of lignin, several cellulose sources have been subjected to alkali treatment using a 2–18 wt% NaOH solution at 30–70 °C for 1–2 hours. In addition to the use of sodium chlorite in a variety of concentrations in an acidic medium, alkaline medium bleaching using hydrogen peroxide/acetic acid was also utilized. Typically, CNCs are prepared by acid hydrolysis with sulfuric acid (64 wt% at 45–60 °C for 30–120 minutes). A variety of acids, including nitric acid, hydrochloric acid, phosphoric acid, and a combination of sulfuric acid and hydrochloric acid, were employed. When isolating nanocrystals, other organic acids and a combination of organic and inorganic acids were also used (Lin et al. 2021). The use of cation exchange resins for solid acid hydrolysis acetic acid/phosphotungstic acid (H3PW12O40)/octanol catalyzed hydrolysis and phosphotungstic acid (H3PW12O40) catalyzed solvolysis (Liu et al. 2015; Torlopov et al. 2019). Considering the organic transformations, some researchers, including (Liu et al. 2015) have modified CNCs with precious metals including rhodium. For the model reaction studies of the cyclopropanation of styrene with ethyl diazoacetate, heterogeneous dirhodium(II) (Rh₂) catalysts based on environmentally friendly and sustainable cellulose nanocrystals (CNC) as support material were obtained by ligand exchange between carboxyl groups on the CNC surface and Rh₂(OOCF₃)₄. In a study (Liu et al. 2015), traditional chemical approaches were followed by ultrasonication, cryocrushing, magnetic stirring, etc. (Liu et al. 2015), and other chemo-mechanical techniques (Kaur et al. 2018; Kaur and Singh 2020). Researchers have reported and on different types of mechanical treatment approaches including ultrasound-assisted methods, including high-intensity ultrasonication (Pandi et al., 2021), combination enzymatic treatment/acid hydrolysis, high pressure homogenization, and cooking with active oxygen and solid alkali (CAOSA) (Wang et al., 2019), and others for the extraction of nanocellulose.

Properties and Surface Modification of CNCs

The adaptability of cellulose nanocrystals is attributed to their functionalization, which can be accomplished in a number of ways. Sulfates, phosphates, and acetates can all be made through straightforward functionalization utilizing sulfuric, phosphoric, and acetic acids. The ammonium persulfate synthesis of recycled medium density fiberboard fibers into carboxylated rod-like cellulose nanocrystals yielded nanoparticles with average dimensions of 170–365 nm and 13–17 nm, and an aspect ratio of 13–21 (Khanjanzadeh and Park 2021). The synthesis of the rod-like acetylated cellulose nanocrystals with diameter and length of 12–18 and 191–234 nm was achieved in a single step by hydrolyzing a mixture of sulfuric acid and acetic acid, which resulted in the esterification of surface hydroxyl groups and the simultaneous hydrolysis of amorphous regions of cellulose. High-crystallinity, rod-shaped, bifunctional CNCs were produced in a mixed acid system containing sulfuric and formic acids (Wang et al. 2021) Applications in composite manufacturing use

3-aminopropyltriethoxysilane (APTES)-functionalized CNC tools as reinforcing elements (Khanjanzadeh et al. 2018). As a result of a condensation reaction between hydroxyl and silanol groups, APTES was adsorbed onto CNC following hydrolysis in water via hydrogen bonds and to the surface of CNC via Si–OC interactions, all without disrupting the crystalline structure of either material. Functionalized cellulose nanocrystals (CNCs) were grafted onto 3-methacryloxypropyl-trimethoxysilane (-MPS) (fCNC). As reported by Yu et al. (2019), fCNC/acrylic PSA films were synthesized via in situ semi-continuous seeding emulsion polymerization by incorporating acrylic pressure-sensitive adhesive (PSA) with varying contents of CNC or fCNC, which improved the compatibility and dispersion of fCNC in the acrylic copolymer and allowed for its use in adhesives and coatings. Some most common functionalization approaches are depicted in Scheme 1.

The production of highly efficient functionalized CNCs by using p-toluenesulfonic acid-catalyzed deep eutectic solvent and a microwave-solvothermal technique resulted in a high yield of 87.6% and provided a high charge density, crystallinity, improved thermostability, and dispersibility (Lu et al. 2021). Improving energy efficiency in the mass production of FCNs allowed for the creation of FCNCs with reinforcing potential on gelatin for nanocomposite application; PDES could be reused, and PTSA and formic acid could be efficiently recovered through recrystallization and evaporation. Phosphoric acid hydrolysis, both heterogeneous and homogeneous, yielded ester derivatives bearing phosphoryl side groups via phosphorylation (Kokol et al. 2015). CNC-based nanosystems with a chitosan (CS) derivative (CNCs/FA-CS-FITC) were found to have high affinity toward folate receptor-positive cancer cells with improved cellular internalization (Pinto et al. 2021). This means that they can be used as nanocarriers with imaging properties for active targeted therapy. Cellulose



Scheme 1 A schematic representation of the numerous functionalization approaches that have been employed in order to manipulate the surface characteristics of cellulose nanocrystals

nanocrystals (CNCs) can have their hydrophilic groups converted to alkyl groups through a nucleophilic substitution process with an alkyl bromide. This change improved dispersion in a nonpolar environment and increased hydrophobicity, both of which have uses in food packaging, and strengthened the PLA's thermal stability, mechanical, and gas barrier qualities (Lee et al. 2020). Esterification of cellulose nanofilms with salicylic acid and p-aminobenzoic acid yielded UV-shielding materials with UVB-shielding characteristics of 99.7% and an ordered cholesteric organization displaying birefringence following evaporation-induced self-assembly (Ling et al. 2020). CNC was dialdehyde and dicarboxylated functionalized at the C-2 and C-3 locations of the glucose using a selective chemical oxidation procedure (Chen et al. 2016). The surface of the sulfated CNC is modified in aqueous media by acrylonitrile, which is then followed by the hydrolysis of grafted nitrile groups. These groups are able to undergo additional directional alterations and can take part in a wide variety of processes (Torlopov et al. 2019).

Inorganic Functionalization of CNCs and Catalytic Applications

Thermal stability, regulated miscibility, and low volatility are just a few of the physical and chemical characteristics of heterogeneous catalysts that contribute to their ease of recovery and recycling. Better selectivity, high conversion, easier synthesis procedures, and low cost have all piqued the attention of researchers in heterogeneous catalysts. Nanocellulose allows for a versatile production platform for these catalysts. Because of their remarkable stability in water, CNCs are particularly suited for catalysis. Catalytic conversions of substrates soluble in solvents that result in a biphasic liquid–liquid system with water are considerably simplified as a result. Since the catalyst is no longer considered a solid support, recycling it requires far less work when using this method.

Because CNCs have hydroxyl groups and sulfonate half ester functions on their surfaces, they make good substrates for the direct deposition and attachment of metal nanoparticles. This is because metal nanoparticles are very small. To create NPs, metal salts are typically fused with a colloidal aqueous solution of CNCs. The material's reactivity can be reduced with the help of various chemical reductants. Silver (Lokanathan et al. 2014), gold (Chen et al. 2015), and platinum (Zhou et al. 2012) nanoparticles can be extracted from CNCs using sodium borohydride as a common reagent. It has been proven that dihydrogen and hydrazine hydrate work effectively. It is fascinating that CNCs can transform metal salts into NPs. The CNC's surface primary hydroxyl functionalities acted as a reducing agent for H₂PtCl₆ in a mixture of water and supercritical CO₂ (100 °C, 100 pressures), resulting in the formation of Pt NPs with diameters ranging from 5 to 30 nm during a procedure in which ultrasmall Ag NPs (1.3–0.3 nm) were created in situ from the oxidation of bulk Ag wire.

Given that water being the most common solvent and the best solvent for suspending CNCs, functionalization techniques based on aqueous solutions have proven to be the most popular. Many approaches are being taken to the study of

functionalization. Reports of CNC functionalization in an organic media have also been made (Lam et al. 2012). It has been suggested that mechanically forcing the composite mixture (which typically takes place in the solid phase) can activate or maintain chemical reactions that functionalize the surface of CNCs. The integration of metal nanoparticles (NPs) is made possible by dry milling CNCs in the presence of ascorbic acid, which dissolves the Ag and Au salts on the surface of the CNCs in the solid phase. NPs with diameters of 6–35 nm and 18–24 nm, respectively, create strong hydrogen bonds with the surface hydroxyl groups (Eisa et al. 2018).

A heterogeneous catalyst made of nanocellulose and hydroxyapatite may catalyze the cyclocondensation reaction between semicarbazide and an aromatic aldehyde at room temperature. Pseudo wet impregnation technique was used in the manufacture of this catalyst. The reaction conditions generated by the NCC/HAp composite catalyst are both cost-effective and low-impact because of the catalyst's recyclable nature (Moodley et al. 2020).

Acetylation with anhydrous acetate yielded the functionalized (acetylated) nanocellulose with an increase in the number of active groups, surface area, and catalytic efficiency. This was achieved through the generation of the nanocellulose-utilizing acetylation. This procedure was performed so that nanocellulose might be created. Acetylated nanocellulose was impregnated with 30-nm-sized nanomagnetite Fe_3O_4 to produce a robust base. Using the produced nanocellulose Fe_3O_4 nanocomposites as a catalyst for the oleic acid to methyl ester synthesis resulted in a conversion yield of 89.21% and a low activation energy of 16.56 kJ/mol. Vegetable oil and other oils rich in fatty acids can be converted into biodiesel with the help of this catalyst (Helmiyati and Anggraini 2019).

2-Aminopyrimidine nanocellulose-supported palladium nanoparticles (CNC AMPDPd) were synthesized as an efficient and reusable heterogeneous bio supported nanocatalyst for the production of coumarin derivatives, a class of antioxidants with a long history of use (Miroosanloo et al. 2018). To obtain the CNCAMPDPd nanocatalyst, nanocellulose crystals were first tosylated (CNC-Tos), then reacted with 2'-amino pyrimidine (AMPD), and finally PdCl_2 solution was added. This process is useful in the production of coumarins, which have unique applications in the pharmacological, pharmaceutical, agricultural, and perfume industries.

In order to achieve covalent enzyme immobilization, dopamine was utilized as a nanocarrier in conjunction with magnetite nanoparticles ($\text{DA/Fe}_3\text{O}_4\text{NPs@CNCs}$). Nanobiocatalysts that combine oxygen/amine functionalities with a high magnetization value and a high specific surface area have been created to increase biomass conversion yield by 20–76% (Ariaenejad et al. 2021). The dopamine-induced polyethylene glycol functionalization of cellulose nanocrystals enhanced their mechanical, barrier, and poly(poly (L-lactic acid) crystallization properties (PLLA). Balanced strength, 168% improvement in ductility, and a 66.4% reduction in the oxygen permeability coefficient were all achieved when CNCs were functionalized with PEG with a lower molecular weight, which was established previously by various studies (Li et al. 2019).

Nanocrystalline cellulose sulfuric acid (s-NCC), a bio-based solid acid catalyst, was synthesized by reacting rod-shaped nanocrystalline cellulose with chlorosulfonic acid. In order to synthesize polyhydroxy pyrimidine-fused heterocycles (PPFHs), this catalyst has been utilized in a pseudo-four-component condensation process of barbituric acid, carbohydrates, and (hetero)aromatic amines in refluxing ethanol (Nikoofar et al. 2018).

Silver NP catalysts were stabilized by using nanocellulose as a support/carrier in combination with hexadecyl-trimethylammonium bromide (CTAB) surfactant. In comparison to the control Ag NPs and CNC/Ag samples, the CNC/CTAB/Ag nanohybrid composite, in which the Ag NPs are immobilized on CTAB-adsorbed CNC, was found to be more effective in converting 4-nitrophenol to 4-aminophenol. This points to the possibility of its catalytic use in wastewater treatment (An et al. 2017).

A one-pot solvothermal approach was utilized in order to create magnetic hollow CuFe_2O_4 nanoparticles (H- CuFe_2O_4 NPs) in situ on the surface of CNC. These nanoparticles had consistent sizes and were hollow. As compared to CNC-based ferrite catalysts, H- CuFe_2O_4 /CNC has demonstrated stronger catalytic activity in the reduction of 4-nitrophenol in aqueous solution, with a higher rate constant of 3.24 $\text{s}^{-1}\text{g}^{-1}$ (0.50 $\text{s}^{-1}\text{g}^{-1}$). With their one-of-a-kind hollow mesostructure and CNC introduction, H- CuFe_2O_4 NPs show promise as a catalyst thanks to their high conversion efficiency (even after 10 cycles), low environmental effect, and good magnetic separability (Zhang et al. 2018).

Composites of manganese dioxide (MnFe_2O_4) and cellulose nanocrystals (MnFe_2O_4 /CNC) were developed, and they exhibited desirable qualities such as a high specific surface area, a minuscule particle size, and a narrow band gap. These properties might be used in a variety of applications. More than 60% quicker breakdown of methylene blue (MB) pollutants was catalyzed compared to MnFe_2O_4 . The high magnetic properties and robust catalytic activity of MnFe_2O_4 /CNC composites during recycling may make them beneficial in the clean-up of polluted areas (Zhan et al. 2018). A zerovalent iron (ZVI) nanocatalyst supported on bamboo pulp cellulose nanocrystals (CNCs) was able to degrade methylene blue in a single step. A component of next-generation nanomachines for sensing, imaging, and drug delivery, it has been shown to be active in the hydrogenation of 4-nitrophenol into 4-aminophenol. CNC-supported ZVIs are a viable option for next-generation nanomachines utilized in sensing, imaging, and drug delivery since they can move independently in the presence of peroxide fuel and their speed and trajectory may be modified remotely (Dhar et al. 2015).

The C–N coupling reactions of open-chained and cyclic secondary amines and electron-deficient vinylic compounds can benefit from copper nanoparticles supported on nanocellulose that have an average size of 7 nm, and this size range was achieved through a simple chemical reduction process. There was a wide range of yields, from 82% to 95%, and the heterogeneous catalysts could be recycled up to six times without losing much of their catalytic activity (Goswami and Das 2018). Producing and using copper nanoparticle catalysts based on extremely crystalline nanocellulose allowed for the regioselective synthesis of 1,4-disubstituted 1,2,3-

triazole from the 1,3-dipolar cycloaddition of various azides and alkynes. The returns were anywhere from 68.0% to 99.0%. Through five iterations, its catalytic efficiency remained at roughly 90%. Several researchers (Chetia et al. 2017) came to this conclusion.

Adsorption of iron oxide nanoparticles can be accomplished using microcrystalline cellulose that has been functionalized with chlorosulfonic acid and cellulose using the co-precipitation technique developed by Massart. Magnetite-decorated sulfate cellulose nanoparticles (MDSCNs) are a biocompatible magnetic composite with high adsorption efficiencies (up to 90%) in water filtration (De Nino et al. 2020). Controlled and selective room temperature conversions of sulfides to sulfoxides and primary alcohols to aldehydes were achieved using copper nanoparticles immobilized on nanocellulose. High yields of sulfoxides were produced from aromatic, aliphatic, and heterocyclic sulfides, while no over-oxidized sulfones were formed during the oxidation of alcohols to aldehydes (Dutta et al. 2019). By reacting 1,8-diaminonaphthalene with various aldehydes at room temperature in the absence of solvents, a green, bio-based, eco-friendly, and recyclable catalyst, $\text{Fe}_3\text{O}_4@\text{NCs}/\text{BF}0.2$ (BF is BF_3OET_2), was used to synthesize 2,3-dihydro-1H-perimidine derivatives. The reaction proceeded quickly, yielded high-purity products, and had minimal environmental impact (Mirjalili and Imani 2019). Heterogeneous dirhodium(II) catalysts based on cellulose nanocrystals (CNC-Rh_2) were produced through ligand interaction between carboxyl groups on the CNC surface and $\text{Rh}_2(\text{OOCF}_3)_4$. Immobilization of CNC-Rh_2 catalysts via covalent bonding, in combination with the separation of binding positions and active sites of CNC-Rh_2 , provides a high stability against leaching and permits the recovery and reuse of the catalyst during the cyclopropanation reaction, where it was found to perform well in a model reaction (Liu et al. 2015).

Catalytic Transformation of Organic Compounds for Environmental Purposes

The unique properties of nanocellulose, such as its bioavailability and ability to dissolve in water, have been the subject of a great deal of research and development over the past few years. Recent studies have also shed light on how these state-of-the-art materials perform as catalysts for a wide variety of reactions, such as reduction, oxidation, coupling, and electrocatalytic reactions. These complex nanocomposites are built for a wide variety of uses, one of which is safeguarding the natural world. It has been shown that sulfonates, carboxylates, and hydroxyls frequently serve as binding sites for metal catalysts. The nanocelluloses are considered a support platform upon which the metal catalyst can be immobilized.

Research into the use of nanometer-sized cellulose materials functionalized with metallic systems in catalytic transformations of organic compounds is a hot topic in the field of sustainable and environmentally friendly products made from natural resources like cellulose. CNC-based materials have been used in a variety of cutting-edge applications, including as adsorbents, catalysts, photocatalysts, flocculants, and

membranes for environmental remediation, in addition to more traditional uses like reinforcing plastics and papers.

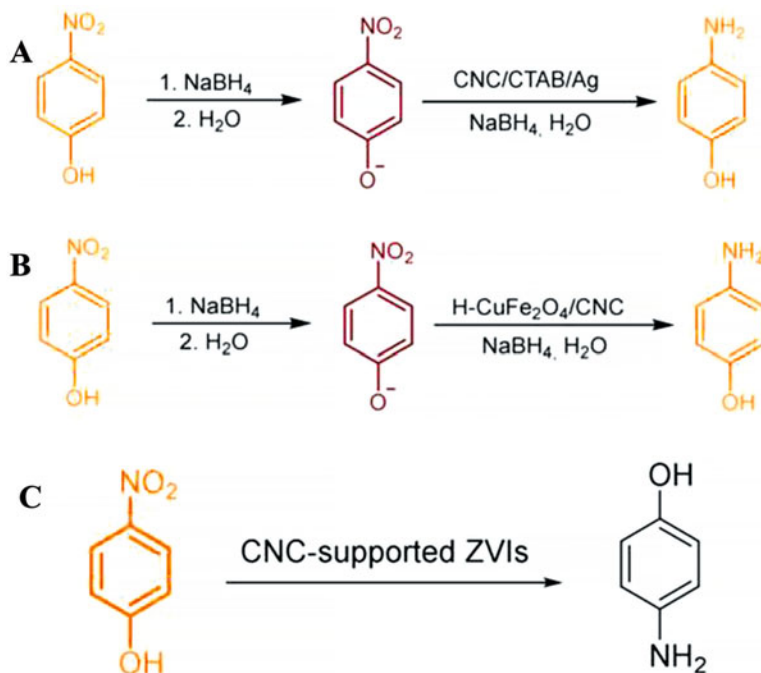
Reduction Reactions

In an aqueous solution, hexachloroplatinic acid was reduced by cellulose nanocrystals, resulting in platinum nanoparticles with a mean diameter of 2 nm. Reduction reaction in a carbon-black support (Vulcan XC-72R), followed by removal of the cellulosic material by heating in air, led to the formation of carbon-black-supported Pt nanoparticles that were highly active toward electrocatalysis of the oxygen reduction reaction (Johnson et al. 2011).

Over a new Pd catalyst that has been chemically bonded to the surfaces of cellulose nanocrystals, Ullmann reactions have been carried out (CNC-BIA-Pd). The catalyst's TON (490) and TOF (1960) values were exceptional, and they displayed high levels of selectivity and activity in a brief reaction time. It was determined that the nanocatalyst was sustainable as a green catalyst because it could be reused for at least eight cycles. The new catalyst was synthesized using 2-(1H-Benzo[d]imidazol-2-yl) aniline (BIA), tosylated cellulose nanocrystals (CNC-TOS), and PdCl₂. When compared to commercial palladium catalysts, the CNC-BIA-Pd catalyst showed superior catalytic efficiency for the Ullmann C–O reaction (Seyednejhad et al. 2019).

In order to reduce 4-nitrophenol, a simple and easy synthesis method was utilized. This method was based on the solid-state production of silver and gold nanoparticles supported on CNC. These nanoparticles have been referred to as Ag@CNC and Au@CNC. This method was used as a sustainable heterogeneous catalyst. Because of its nanoscale nature and its amenability to functionalization via its abundant surface hydroxyl and sulfate groups, CNC is an excellent support. These hybrid CNCs, which can function as heterogeneous catalysts, were synthesized by milling freeze-dried CNC in ambient conditions with the appropriate precursor salt and a simple organic-reducing agent, ascorbic acid. Standalone films of Ag@CNC and Au@CNC were used as a solid catalyst for the reduction of 4-nitrophenol (4-NP) (Eisa et al. 2018).

Reduction of 4-nitrophenol to 4-aminophenol has been reported using a wide variety of metal-decorated CNC catalysts. These include a CNC/CTAB/Ag nanohybrid composite, H-CuFe₂O₄ Nps supported in the presence of CNC, CNC-supported zerovalent iron nanoparticles (ZVIs), etc. (Scheme 2). Reduction of 4-nitrophenol was accomplished with NPs made of Au, Ag, Pd, Au–Pd, Pt, Cu, Ni, and CuO that were supported on CNCs or CNFs. Compared to metal NPs supported by mesoporous silica or other polymers like poly(methyl methacrylate), metal NPs supported by CNC are often more efficient and offer superior TOFs (PMMA). This is because CNCs have the ability to suspend and facilitate diffusion, unlike porous supports, and because of their intrinsically small nanodimensions, CNCs are able to stabilize extremely small metal NPs (5–10 nm in width). Tam and coworkers published the highest TOF for agricultural NPs among Au, Pd, and Cu NPs.

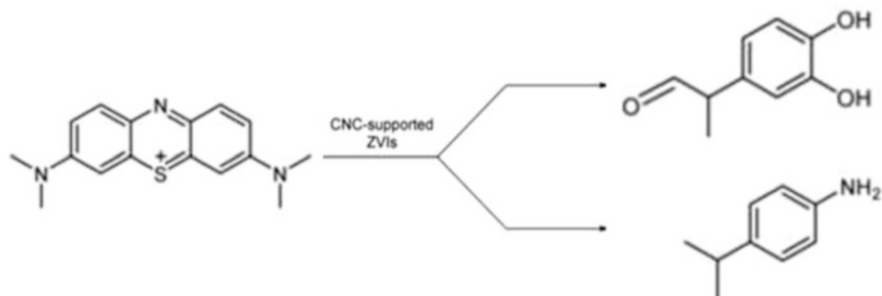


Scheme 2 Catalytic reduction of 4-nitrophenol to 4-aminophenol over various CNC-functionalized catalysts: (a) CNC/CTAB/Ag nanohybrid composite, (b) $\text{H-CuFe}_2\text{O}_4$ Nps supported in the presence of CNC, and (c) $\text{CNC-supported ZVIs}$

Higher TOF values were observed for Pt NPs around 2 nm (2 nm) compared to Pt NPs around 21 nm (21 nm), demonstrating that NP size has an impact on the TOF due to the higher specific surface area and defect content of the smaller NPs (Xiong et al. 2011).

For the catalytic decomposition of methylene blue, a magnetic composite of MnFe_2O_4 and cellulose nanocrystal ($\text{MnFe}_2\text{O}_4/\text{CNC}$) was synthesized. CNC/inorganic nanocatalyst demonstrated enhanced catalytic activity for MB decomposition in H_2O_2 solution compared to that of pure MnFe_2O_4 . In addition, $\text{MnFe}_2\text{O}_4/\text{CNC}$ showed excellent recovery after incorporating a magnet into the reaction system; this bodes well for its application in wastewater treatment. Similarly, zero-valent iron nanoparticles (CNC/ZVI) supported by a CNC have found use in a wide range of settings. Decomposing MB is one application of CNC/ZVI catalyst in wastewater treatment (Dhar et al. 2015). Through the use of UV-visible absorbance analysis, the decomposition was shown to be quantitative. The GC-MS-suggested hypothesized decomposition products are shown in Scheme 3.

It is important to reduce olefins, carbonyls, alcohols, and arenes in academic as well as industrial laboratories in order to increase the atom economy by moving away from stoichiometric quantities of reductant and to find “greener” reaction conditions (according to the 12 principles of green chemistry). In order to do this,



Scheme 3 A general hypothesized decomposition pathways of methylene blue over CNC-supported ZVI nanocatalysts

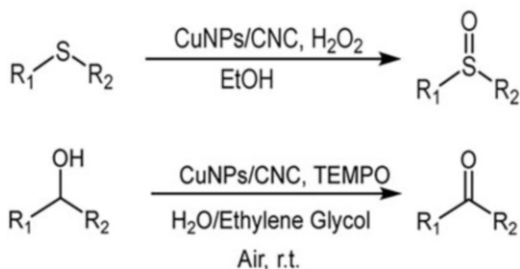
it is necessary to move away from stoichiometric quantities of reductant (Elsevier and de Vries 2007). Employing a metal catalyst and hydrogen gas as a reductant offers a solution to address these challenges and allows recyclability over many cycles of the reaction and, in some situations, greater enantioselectivity with the possible aid of CNC. Pd NPs supported onto CNCs were found to be very effective for the hydrogenation of phenols under simple conditions (4 bar H₂, room temperature, and two hours), with up to 90% yield in 24 hours (Cirtiu et al. 2011). The necessary efficiency of CNCs' support could be determined by comparing the collected data to those of other supported Pd NP catalysts, such as Pd NPs/Al₂O₃ or carbonaceous supports. Comparing the two showed that high yields could be obtained with a Pd loading of only 50%. As platinum is a precious metal, this is crucial information. Two hours of enantioselective reduction of prochiral aromatic ketones in water at 4 bar H₂ pressure was reported in subsequent studies (Kaushik et al. 2015a). Cryo-transmission electron microscopy and tomography revealed that the CNCs were a malicious support that acted as chiral inducers, creating a local chiral environment around the active catalytic centers that allowed for enantiomeric excess (ee) yields of up to 65%. Although this result for enantioselectivity was lower than that achieved with grafted chiral inducers, it was the highest ee ever achieved when using a biomass-based support as the sole chiral source (Yasukawa et al. 2015). For the reduction of aromatic aldehydes, alkenes, and alkynes, CNC-supported Ag NPs have been shown to be effective alongside Pd NP-based catalysts, albeit under more extreme conditions, such as under 40 bar of H₂ and at 100°C for 24 hours (Kaushik et al. 2016). Furthermore, Ru NPs were used for the extremely unfavorable hydrogenation of arenes with H₂ under mild circumstances (4 bar of H₂, ambient temperature, and 24 hours) to achieve full conversion into the saturated ring product (Kaushik et al. 2015b). Styrene and acetophenone were also investigated under the same conditions, with yields toward the fully reduced ethylcyclohexane product being 96% and 76%, respectively, expanding the applicability of the reduction reactions of metals supported on CNC supports. Interestingly, 2-acetylfuran, a heterocyclic aromatic compound, yielded a superb 90% in tests.

Oxidation Reactions

Oxidation processes are fundamental to the functioning of the entire natural world and are necessary for the production of organic compounds. Oxidation reactions are commonly used in the petrochemical industry. Molecular oxygen, hydrogen peroxide, osmium tetroxide, and other oxidizers are used. By eliminating the excess gas produced during a reaction, using traceless gaseous reagents instead of chemicals allows for the creation of greener processes by reducing the amount of waste generated during the purification process. The synthesis of organic compounds relies heavily on oxidation processes, which add new functional groups to molecules or modify existing ones. Metal stoichiometric oxidants are widely used in processes, but they go against the 12 principles of green chemistry because they create massive amounts of waste (Védrine 2019). In a similar fashion, many conventional techniques for oxidizing alcohols and sulfoxides with toxic and environmentally harmful stoichiometric oxidants like hypochlorite, permanganates, chromium oxides, and others are already available (R O C Norman and Coxon 1993). Catalysts are absolutely vital for activating these oxidants, but non-waste-producing oxidants like air, O₂, or H₂O₂ provide support for environmentally acceptable and moderately cheap systems (Noyori et al. 2003). Heterogeneously catalyzing oxidation processes can be done with any number of different types of metals and metal oxides, including those of common metals, noble metals, and mixed metals. It is impossible to overstate the significance of these supported metal catalyst-catalyzed reactions. The fact that these are heterogeneous catalysts for oxidation processes does not change the fact that they are cumbersome and have a weak support system. As a biodegradable, nontoxic, renewable, and compact support for disseminating metal-based oxidation catalysts, CNC presents significant opportunities.

For instance, CuNP/CNC catalytic material was studied as a heterogeneous catalyst for the oxidation of sulfides under mild reaction conditions, with good yields and in a controlled amount of time. There were CuNPs-immobilized on nanocellulose. Sulfides are versatile substances with significant pharmacological activity that can be found in nature (Paonessa et al. 2018). There is little difficulty involved in making them. Using the nitroxyl radical TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy), the CuNP/CNC catalyst facilitated the oxidation of a range of alcohols (Scheme 4). The high potential of copper is likely responsible for the high yield (82–95%) achieved by the

Scheme 4 On op, CuNP/CNC oxidizes sulfides in ethanol medium, while on the bottom, CuNP/CNC and TEMPO-mixed composite system oxidize a variety of alcohols – both R1 and R2 can be aryl or alkyl systems



heterogeneous catalyst, which can be easily recovered from the reaction mixture and reused multiple times with little to no loss of activity. Catalyst was recovered by filtration, washed with water and acetone, and then reused in subsequent runs with identical conditions and a new supply of substrates.

Grafting copper-tetrasulfonate phthalocyanine onto cellulose nanocrystals made them a high-yield, heterogeneous, and selective catalyst for the aerobic oxidation of alcohols and alkyl arenes to the corresponding aldehydes and ketones in *o*-xylene at room temperature with good yields and recyclability in an aqueous green solvent. Both the cationic CNC and the anionic sulfonate Cu-PC make extensive electrostatic contact with one another and form strong H-bonds within the catalytic system. To our knowledge, no Cu was lost during the catalytic reaction, and the system was recyclable up to seven times (Chauhan and Yan 2015). For the aerobic oxidation of primary and secondary benzylic alcohols to aldehydes and ketones at room temperature, it was found that cobalt(II) complexed with functionalized nanocellulose crystals made of ethylenediamine was a highly efficient heterogeneous catalyst. This heterogeneous catalyst was stable enough to be recycled more than five times. It was possible to achieve good catalytic activity with a low loading of Co (II) (1.04 wt%) because of the way cobalt was distributed on the high surface area nanocelluloses (Shaabani et al. 2014).

Coupling Reactions

C–C- coupling reactions have been a mainstay in conventional pharmaceutical procedures for a very long time. These reactions are used to synthesize active pharmaceutical ingredients (APIs) and drug molecules. As a consequence of these and other early successes with Pd-catalyzed cross-coupling reactions, Suzuki, Negishi, and Heck were awarded the 2010 Nobel Prize in Chemistry. From the early works of Tsuji and the formation of allylmalonate via an allylpalladium analog (Tsuji et al. 1965) to the studies of Heck (1968a, b) and Mizoroki (1971) demonstrating carbon–carbon bond formation with palladium catalysts in the late 1960s, these reactions have a long and illustrious history.

Nanocellulose is useful for binding not only nanoparticles but also metal ions. This principle is illustrated by the synthesis of a heterogeneous nanocellulose–palladium nanoparticle catalyst for Suzuki cross-coupling reactions in water. Chemically modifying cellulose with coupling agents enabled an interface to create a chemical bridge between the reinforcement and matrix, which can be used for chelating or adsorbing heavy metal ions. Dehydrated cellulose with surface hydroxyl groups and amino groups that bind heavy metal anions improves the adsorption capacity, as does aminopropyltriethoxysilane (APS), a silane coupling agent bearing one amino group in one molecule that can be hydrolyzed to the silanol group. Cd(II), Cu(II), and Ni(II) were removed from polluted aqueous solutions using aminosilane (APS)-modified nanostructured microfibrillated cellulose (MFC) with experimental maximum adsorption capacities ranging from 2.72 to 4.20 mmol/g and an optimal pH value of 5 (Hokkanen et al. 2014). A variety of organic coupling

reactions, such as the Heck coupling, Ullmann coupling, Suzuki coupling, and A3 coupling (Scheme 3), have been successfully carried out over metal-stabilized nanocellulose nanocomposites. Numerous recycling experiments have been performed in these studies to effectively prove the advantage of CNCs as a support for the recovery of the catalytic species, and they have been used in a wide range of different coupling reactions involving Pd NPs. Examples of coupling reactions include the multicomponent coupling reaction of aldehyde, amine, and alkyne (A3). Using benzaldehyde, piperidine, and phenylacetylene as model substrates and 80 °C and 24 hours with 4.4 mol% catalyst loading, Huang et al. conducted the A3 coupling to high yield (Huang et al. 2013).

In organic transformations, multicomponent coupling reactions are given special consideration. High yields can be obtained after 24 hours of treatment at 80 °C with Au NPs supported on thiol-functionalized CNCs in a conventional aldehyde-amine-alkyne (A3) coupling. Benzaldehyde, piperidine, and phenylacetylene were used as model substrates, with a catalyst loading of 4.4 mol%. Although the maximum yield was achieved in sterile conditions, there are reports of lower yields when using solvents like water (87%), toluene (92%), and ethanol (56%).

The A3-coupling reaction was carried out by Huang et al. using AuNPs supported on thiol-functionalized CNCs catalysts (coupling of aldehyde-alkyne-amine). The system showed remarkable activity in water and without a solvent, as well as strong promiscuity toward a variety of reactants, when heated to 80 °C for 24 hours. One of the catalyst's best qualities was its high recycling rate (up to 11 times) (Huang et al. 2013).

Electrocatalysis

Shen et al. 2022 introduced a sustainable synthesis route that modified the carbon aerogel's (CA) intrinsic chemical nature and produced a hierarchically porous microstructure, making CA a promising new material platform for electrocatalysis. A thermally sacrificial template Cd metal with a low boiling point (767 °C) and TEMPO-mediated oxidized cellulose nanofibers (TOCNFs) was used to assemble a TOCNFs-Cd²⁺ hydrogel/aerogel, which upon pyrolysis at a moderate temperature was converted to ultralight N-doped carbon nanofiber aerogel (N-CNA-Cd). Due to their abundant interior micro- and mesoporous, rich defects, enormous BET-specific surface area (1782 m² g⁻¹), and interconnected fibrous network, N-CNA-Cd samples have shown electrocatalytic efficacy toward oxygen reduction reaction, a large electrochemical active surface area, and considerable stability under both alkaline and acidic conditions (Shen et al. 2022). Using electrostatic interaction and a specific Co–N interaction, a uniform coating of the cobalt–adenine coordination polymer (CoA) complex was achieved on a hydroxyl-rich and negatively charged CNC self-template. Due to CNC's role in facilitating the well-dispersed Co nanoparticles and the formation of the unique bimodal micro-/mesoporous structured carbon, the carbonized CoA@CNC exhibits appealing bifunctional electrocatalytic activity for both the oxygen reduction reaction (ORR) and the oxygen evolution reaction (OER).

The CoA@CNC-700 air cathode was used to create a rechargeable Zn–air battery with high-power density (187 mW cm^{-2}), specific capacity (782 mA h g^{-1}), and cycle life (50 h) (Shen et al. 2020). Immobilized powder catalyst nitrogen-doped molybdenum carbide nanobelts (N-Mo₂C NBs)(N-Mo₂C@NCs) exhibit good performance toward the hydrogen evolution reaction. Nanocellulose, which exhibits excellent dispersibility and multiple exposed catalytically active sites, was used in place of Nafion (HER). N-Mo₂C NBs, graphene nanosheets, and nanocellulose (N-Mo₂C/G@NCs) formed a conductive film that could be bent and reshaped while still maintaining its high performance in HER applications (Li et al. 2019).

The production of nanostructured silver has been shown to benefit from the use of reducing agents that are derived from nanocellulose crystals derived from cotton. As part of the procedure, cellulose nanocrystals were suspended in an aqueous solution of AgNO₃ and heated for two hours at 80 °C. Metallic silver was confirmed to have been produced via transmission electron microscopy, UV/Vis, and X-ray diffraction (TEM). The product suspension was used to develop nanocomposite films on a smooth surface. The films' textures changed depending on the ratio of cellulose. Analyzing the electrochemical and electrocatalytic activity of these films with cyclic voltammetry and rotating-disk electrode voltammetry revealed that the nanocomposite films were very efficient electrocatalysts for the reduction of oxygen in alkaline media (Johnson et al. 2010). Since silver's activity is nearly as high as platinum's while being substantially less expensive, it is a superior cathode material for alkaline fuel cells, which use the electrocatalytic oxygen reduction reaction.

Pt nanoparticles were synthesized using hexachloroplatinic acid and subsequently reduced using cellulose nanocrystals derived from cotton in an aqueous solution at mild temperatures. Pt/CNC catalytic systems were characterized by transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS), and thermogravimetric analyses (TGA). Physical and chemical analysis of nanoparticles 2 nm in size showed that they consisted of an oxidized surface layer over a Pt metal core. Producing carbon-black-supported Pt nanocatalyst was doable by carrying out the reaction on a carbon-black support (Vulcan XC-72R) and then burning off the cellulosic material in air. Since electrochemical measurement shows that Pt/C electrocatalysts are very active, especially for the oxygen reduction reaction, this strategy seems like it could be very useful in the production of Pt/C electrocatalysts (Johnson et al. 2011).

Other Types of Catalytic Transformation

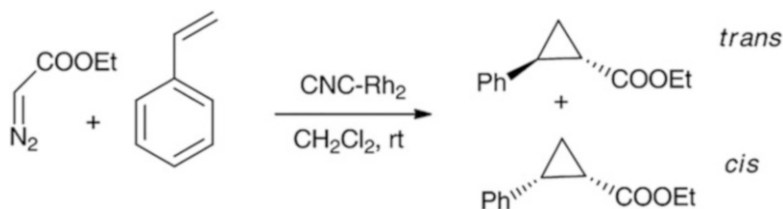
The formation of tetra-sulfonated porphyrin, which was introduced in the subphase as an anionic competitor, strongly inhibited the binding of CNCs to the floating fullerene layer using Langmuir hybrid films that were based on the electrostatic interaction between cationic fulleropyrrolidines deposited at the air/water interface and anionic CNCs dispersed in the subphase. Sturdy hybrid films were assembled thanks to the interfacial interaction between fulleropyrrolidines and CNCs, and these films were then transferred to solid substrates. Anodic photocurrents at 0.4 V vs.

Ag/AgCl were generated by ITO-electrodes modified with five-layer hybrid films, and their intensity (230 nA/cm^2) was four times higher than that observed with the sole fullerene derivative (60 nA/cm^2). Using chronoamperometry, we observed the photo-electrochemical behavior of ITO electrodes coated with five layers of FP or FP/CNCs in a neutral aqueous environment. This behavior included alternating dark and light periods and a significant increase in the light-induced current increment.

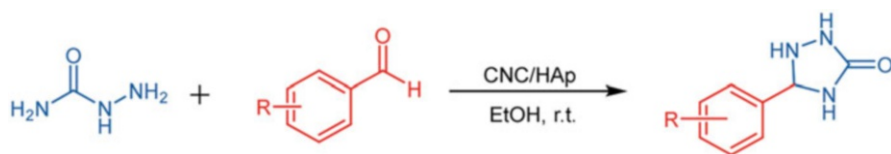
Solid-state ^{19}F and ^{13}C NMR spectroscopy confirmed the formation of heterogeneous dirhodium(II) (Rh_2) catalysts using environmentally friendly and sustainable cellulose nanocrystals (CNC) as the support material. Surface carboxyl groups on CNC interact with $\text{Rh}_2(\text{OOCCH}_3)_4$ to form a ligand exchange complex. This CNC–dirhodium catalyst performed admirably in a model cyclopropanation reaction (Scheme 5), even at extremely low dirhodium(II) surface concentrations (0.2 mmol g^{-1}). CNC-covalent Rh_2 is immobilized through covalent bonding, ensuring a high level of stability against leaching and allowing for the recovery and reuse of the catalyst during the cyclopropanation reaction due to the specific locations of its binding positions and active sites (Liu et al. 2015).

Through an azide–alkyne cycloaddition that was catalyzed by copper (I), Thielemans and colleagues were able to graft an imidazolium salt onto the surface of CNCs. Following this, the feasibility of employing the modified CNCs in ion-exchange processes was investigated. The ion-exchange capacity of modified CNCs was determined by soaking them in a solution of lithium bis(trifluoromethanesulfonyl)imide for an entire night. By using a heterogeneous method to immobilize imidazolium salts, this study paves the way for the development of a variety of catalytic and ion-exchange systems.

Aerogels made from cellulose nanoparticles and membranes made from cellulose nanoparticles are just two examples of the many cellulose nanocomposites that have found successful use in environmental contexts. Zhu and colleagues made nanocellulose aerogels with varying amounts of metal-organic framework (MOF) particles. The CNCs provide structural support for the functional MOFs in a networked material that is both three-dimensionally flexible and lightweight. Changing the proportion of ingredients in the beginning allows for a wide variety of MOF loadings. The ability to incorporate three distinct MOFs into CNC-based aerogels showed not only the versatility of the processing technology and materials, but also



Scheme 5 A simplified model of the heterogeneous catalyst CNC-Rh_2 at work in a cyclopropanation reaction



Scheme 6 Schematic representation of the CNC/HAp-catalyzed synthesis of triazolidine-3-ones via the reaction of aromatic aldehydes and semicarbazide

that the MOFs maintained their crystallinity and functional properties during the incorporation process. The hybrid material was effective in removing Cr(VI) from water that had been contaminated with the heavy metal. When the composite system is in the aerogel form, its crystallinity, porosity, and accessibility are all maintained, making it an excellent adsorbent for research into the purification of water. Aerogels lacking metal-organic frameworks (MOF; controls) were unable to remove Cr after 24 hours (VI). Approximately 85% of Cr was absorbed by the aerogel containing 50% MOF after 24 hours (VI). Cr(VI)abilityability's ability to adsorb material was drastically reduced along with the decrease in MOF concentration. Aerogels with a lower MOF loading (33.3% and 20 wt% of MOF) are able to absorb 67% and 51% of the Cr(VI), respectively (Yuan et al. 2020; Zhu et al. 2016).

Nanocellulose (CNC)/hydroxyapatite (HAp) has been used as a catalyst in the reaction of aromatic aldehydes with semicarbazide, and Moodley and coworkers have achieved excellent yields in this synthesis (Scheme 6). All three types of electron-donating and -withdrawing substitutes (ortho, meta, and para) performed admirably under the reaction conditions and produced the expected products. The mechanism of this reaction is completely understood when the CNC catalyst is taken into account. In the first stage, the carbonyl group of the aldehyde is attacked by the acidic CNC/HAp-protic solvent mixture, which may produce a transient zwitterionic intermediate. Restarting the catalytic cycle requires the removal of water. There was no noticeable degradation of the catalyst after five reuses, indicating the system's stability under reaction conditions (Moodley et al. 2020).

Photocatalytic Transformation of Organics for Environmental Remediation

An increasing number of water pollution remediation projects are employing nanocomposites based on nanocellulose for the catalytic breakdown of organic contaminants. The two most common types of guest NPs used in catalytic applications are precious metals and photocatalysts like TiO₂ (Au, Ag, Pt, etc.). In order to keep nanoparticles from clumping together, catalytic supports like nanocellulose are frequently used. When used in mild reaction conditions, photocatalysis efficiently transforms solar energy into chemical energy for the elimination of pollutants (Shayegan et al. 2018; Anise et al. 2020). The S-doped carbon nanosheet (SCN)/ZnO composite photocatalyst synthesized using the freeze-dried cellulose

nanocrystals (CNCs) as the carbon precursor has a regular nanosheet-like morphology, integrated graphitic structures (ID/IG of 0.692), rich surface heteroatoms (14.62 at% for O, 1.93 at% for S), and increased surface reactive site with specific surface area of 45.55 m² g. In addition to its excellent photocatalytic performance for methylene blue (99%), the SCN/ZnO composite has potential for use in the removal of other organic pollutants from the environment, such as phenol (91%), formaldehyde (92%), acetone (79%), and methyl alcohol (84%). After four cycles, the composite remains stable and reliable. CNCs are able to function as carbon nanosheets because they self-organize into a 2D nanosheet structure during freeze drying due to electrostatic repulsion (Han et al. 2013; Zhang et al. 2021).

Eosin yellow (EY) is a carcinogenic dye that was successfully removed and photocatalytically degraded from aqueous solutions using a conductive conjugated polymer/semiconductor system, poly(pyrroleco-aniline)-coated TiO₂/nanocellulose composite (P(Py-coAn)-TiO₂/NCC) (Fig. 5a) (Anirudhan and Rejeena 2015). Pseudo-second-order kinetics and the Langmuir and Fritz–Schlunder isotherm models indicated that 91.7% of the dye was adsorbed within 90 minutes at a pH of 4.5, and a dose of 3.5 g/L was required for the complete adsorption of EY from aqueous solutions. Polypyrrole and polyaniline were coated onto TiO₂/NCC by chemical oxidation polymerization of pyrrole and aniline with FeCl₃, and TiO₂/NCC was prepared from NC and the precursor Ti(OiPr)₄ via chemical precipitation methods. The adsorption capacity of 3.39 10⁵ mol/g and the degradation of 92.3% of the dye in 90 min under sunlight (Fig. 5b) are indicative of the photocatalytic degradation of adsorbed dye.

In addition, a composite of Ag₃PO₄ and nanocellulose was synthesized with a photocatalytic degradation rate of methyl orange of 90% in DI and 70% in wastewater after the same amount of time under solar irradiation (Lebogang et al. 2019). Adsorption of methylene blue from model water using a CNC/ZnO nanocomposite prepared from sawdust followed the Langmuir isotherm model (Fig. 6) (Oyewo et al. 2020).

TiO₂ nanorods were grown in situ on cellulose nanocrystals, and then decorated with Au nanocrystals to serve as a functional template. To degrade RhB dyes via

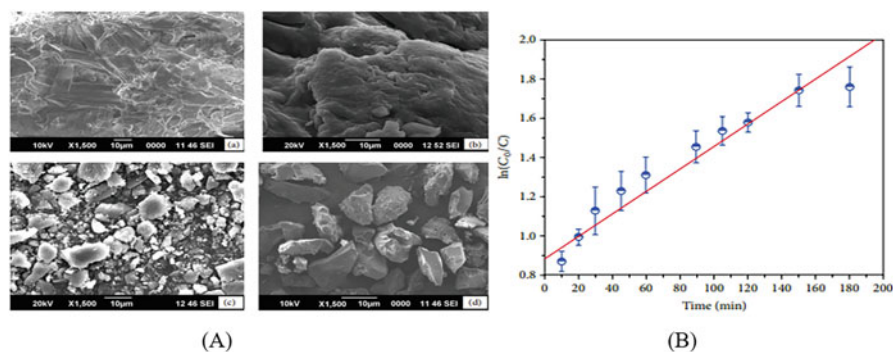


Fig. 5 (a) SEM photographs of (a) cellulose, (b) NC, (c) TiO₂/NCC, and (d) P(Py-co-An)-TiO₂/NCC; (b) kinetic data for the photodegradation of EY onto P(Pyco-An)-TiO₂/NCC

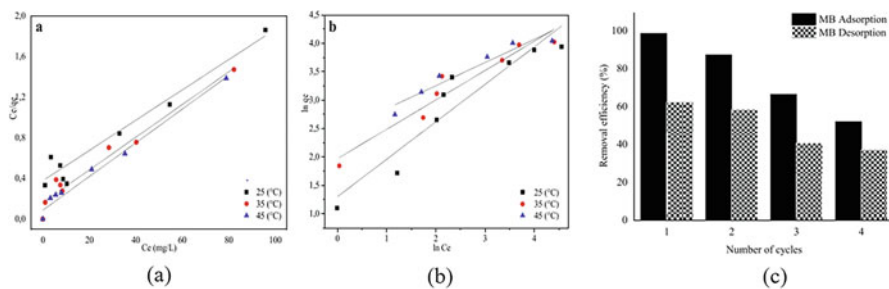


Fig. 6 Fitting of adsorption isotherm models: (a) Langmuir isotherm model; (b) Freundlich isotherm model; (c) adsorption–desorption efficiency of MB using 2.5 M NaCl eluents

photocatalysis in sunlight, a nanostructured hybrid of biological and inorganic NR photocatalyst decorated with Au NCs is employed (Nair et al. 2020). Surface-functionalized cellulose nanocrystals were used to achieve selective adsorption and separation of organic dye mixtures (methylene blue, with either methyl orange or rhodamine b or crystal violet) (Mohammed et al. 2021). In the adsorption experiment, both bare and polydopamine (PD) and melamine-formaldehyde (MF)-coated CNCs were used. Adsorption efficiency for MB on pure CNCs with anionic sulfate ester groups was 85.78%, while adsorption selectivity for MO was 100% on PD-CNCs and MF-CNCs with cationic amine groups. Pesticide contaminants in organic and aqueous media were removed using amine-functionalized cellulose nanocrystals (Swasy et al. 2020). For example, 100% degradation of malathion in organic systems was achieved by using a poly(ethylenimine) cellulose nanocrystal (CNC-PEI), and 95% and 78% degradation of deltamethrin and permethrin were achieved in aqueous systems, respectively (Fig. 7). Therefore, amine-modified cellulose nanocrystals can be a low-cost, high-efficiency material for cleaning up water tainted with pesticides.

C-doped graphitic carbon nitride/tungsten oxide (C-doped $g\text{-C}_3\text{N}_4/\text{WO}_3$) was synthesized by hydrothermal impregnation with cellulose nanocrystal, high-temperature calcination, and electrostatic self-assembly with WO_3 nanocuboids, resulting in a ternary Z-scheme (Zhao et al. 2021). The photocatalytic degradation of 75.0% of tetracycline was achieved in 60 minutes thanks to the high specific areas ($57.20 \text{ m}^2 \text{ g}^{-1}$), C-substitution, and formation of Z scheme heterojunction, all of which resulted in a narrower bandgap, enhanced visible-methyl light absorption and separation of charge carrier, faster interfacial charge transfer, good oxidation/reduction capacities, and the like. Photocatalytic activity was demonstrated in the removal of organic pollutants from wastewater using a nanocomposite film of carboxymethyl cellulose reinforced with cellulose nanocrystals in the presence of bismuth ferrite (BFO)/poly-o-phenylenediamine (PoPD). This included the removal of methyl orange by 94% at 20% wt CNC (Nazri et al. 2021).

Recent research has also concentrated on the reductive environmental remediation of pollutant dyes like methylene blue by using Pd and Fe NPs immobilized on

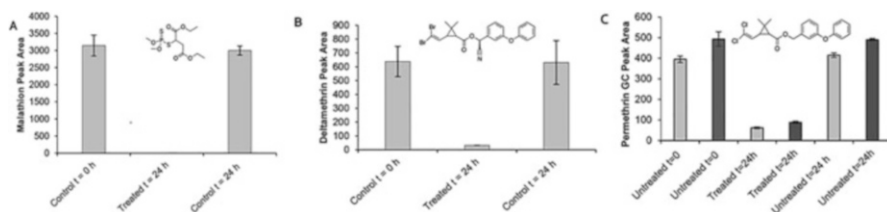


Fig. 7 (a) Degradation of malathion, (b) deltamethrin, and (c) permethrin with CNC-PEI. Untreated samples contain only pesticide solution and serve as controls at $t = 0$ h and $t = 24$ h. Malathion and deltamethrin were conducted at RT while permethrin was conducted at 35°C

CNCs. These NPs have been shown to be an effective catalyst toward this goal. Using CNC-supported metal NPs as a catalyst, NaBH 4 as a reducer, and UV-Vis monitoring, this model reaction has been investigated. The latter case demonstrated CNC-stabilized Fe Nps's possible utility in addressing water pollution.

Mesoporous Fe_2O_3 has been manufactured using sol-gel techniques and used as a catalyst in photocatalytic processes, with CNCs serving as a template. Pore sizes in mesoporous $-\text{Fe}_2\text{O}_3$ varied from 4 to 42 nm. For $-\text{Fe}_2\text{O}_3$, the crystalline size was around 57 nm, and the specific surface area was $47.26\text{ m}^2\text{ g}^{-1}$. Methylene blue was photodegraded with the help of an $-\text{Fe}_2\text{O}_3$ catalyst that was synthesized in the lab. The results demonstrated that the photocatalytic activity of $-\text{Fe}_2\text{O}_3$ on CNC templates was superior to that of the samples prepared without CNCs (Liang et al. 2015).

Conclusions and Future Perspectives

As the study of nanocellulose-supported catalysis progresses, it is becoming clear that there is the potential for the field to become a source of solutions to societal problems that are more easily accessible, reasonably priced, environmentally friendly, dynamic, selective, and harmless. Nanocellulose (NC) is rapidly gaining attention in the fields of nanocomposites and sustainable materials. NC has many applications outside of the medical industry, including biodegradable materials, reinforcing agents, packaging films, permeable membranes, and even biodegradable packaging. NC functionalized with organic and inorganic groups has proven useful in a wide variety of applications, including as a catalyst in chemical reactions. By mimicking the structure of a conventional heterogeneous catalyst, nanocellulose allows for the development of soluble and suspendable catalytic systems, typically for use in water. Even though prefunctionalization to provide anchoring groups has also been extensively discussed over the years, direct functionalization with metal nanoparticles is ideally suited to the surface chemistry of nanocellulose. It is argued that the resulting catalytic systems are powerful catalysts for a range of processes.

The Heck, Ullmann, Suzuki, and A3 couplings are examples of significant reactions that proceed with high yields. Other significant reactions include reduction

reactions that have been accomplished with catalysts such as Au, Ag, Pd, Au–Pd, Pt, Cu, Ni, and CuO NPs supported onto CNCs or composite catalysts such as CNC/CTAB/Ag nanohybrid composite, H-CuFe₂O₄ Nps supported in the presence of CNC or CNC-supported zero environmental remediation, electrocatalysis, oxidation reactions, photocatalytic transformation of organics, etc. The efficiency of nanocellulose-based catalysts relies on the ability of such support to retain very small metal NPs while promoting good mass diffusion through the catalyst. These advancements have allowed for high TOFs and even enantioselective induction. Therefore, many methods of inorganic functionalization of nanocellulose can be used in various types of catalysis. Electrocatalytic reactions, oxidation, reduction coupling, and other processes are all possible with these CNCs-based nanocatalytic systems. These reactions have applications in catalysis, particularly electro- and photocatalysis.

Emerging nanoparticles of a wide variety of other polysaccharides are expected to be taken into consideration as potential support for novel catalysts in the not too distant future. In particular, nanocrystalline chitin, chitosan, and starch nanoparticles (SNPs) provide access to the amiable nitrogen manifold and present an opportunity to supplement the chemistry of cellulose. The development of such nanocatalysts has been facilitated by recent examples. It is concluded that the utilization of nanocelluloses in catalytic applications has resulted in the development of novel, intriguing, and environmentally friendly catalytic systems. We hope that new uses will emerge as a result of these discoveries.

For catalytic purposes, smaller metal NPs are preferable, while larger NPs are required for SERS. The loading of NPs is also important for the practical application of nanocomposites. It is important to learn how much different NPs can weigh down a nanocellulose matrix. Nanocellulose's long-term viability could be jeopardized by the presence of inorganic components in the matrix. It is important for scientists to work on rejuvenating or extending the life span of nanocomposites to ensure their continued usefulness. Nanocellulose-based nanocomposites may be an option when looking for a low-cost, eco-friendly material to combat growing environmental concerns.

Aerogels, membranes, and other composite materials based on nanocellulose have been utilized in the treatment of wastewater and waste oil, respectively. The primary applications of such functionalized CNC materials included oil–water separation, catalytic degradation of organic contaminants, adsorption of heavy metal ions, and adsorption of colors. While the results of many scientific experiments have been encouraging, the difficulty of scaling up for industrial applications remains a major issue.

Aggregation or agglomeration of CNC is a common problem in any types of nanocomposite systems, making it difficult to achieve uniform dispersion and distribution of inorganic system in to CNC matrix. Incorporating inorganic system into CNC efficiently requires a unique chemical modification process. It is expected that advances in nanotechnology involving renewable nanomaterials like CNC will yield technologically advanced, non-polluting, and above all biodegradable catalytic materials.

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Bacterial Nanocellulose (BNCs) Supported Inorganic Nanomaterials for Catalytic Applications

33

Krishnakumar Melethil, Sharon Varghese, Albin James, M. H. Rubiya, and Bejoy Thomas

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K. Melethil · S. Varghese · B. Thomas (✉)

Department of Chemistry, Newman College, Thodupuzha, Kerala, India

e-mail: bejoy.thomas@newmancollege.ac.in

A. James

Mahatma Gandhi University Centre for Research in Chemistry, Newman College, Thodupuzha, Kerala, India

Mahatma Gandhi Centre for Teacher Education, Thodupuzha, Kerala, India

M. H. Rubiya

Mahatma Gandhi University Centre for Research in Chemistry, Mar Athanasius College, Kothamangalam, Kerala, India

Abstract

There is an increasing interest in the field of nanocomposites and sustainable materials, namely, for catalytic and biomedical applications, and bacterial nanocellulose (BNC) is an interesting and renewable natural nano-biomaterial that could play a role in these areas. The exceptional crystallinity, mechanical strength, purity, porosity, moldability, water-holding capacity, biodegradability, and biological affinity of BNC are only a few of its remarkable physical features. These properties distinguish BNC as an excellent substrate/matrix for customizing with metals, metal oxides, or metal compounds/complexes, as opposed to plant-based nanocellulose. The low rate of catalyst leaching from the catalyst support and the simplicity of separating the catalyst from the reaction medium provide cellulose-supported catalysts a high recycling potential. These bio nanocomposites based on BNCs have many uses, one of which is in catalysis. These BNC-supported metal based catalysts have a wider range of applications, including, environmental purification, bioremediation, the treatment of wastewater, photocatalysis, electrocatalysis, various organic transformations including coupling processes, reduction and oxidation reactions, and a great deal more. Novel catalytic applications based on the insights on metal supported BNC systems are expected to emerge eventually.

Keywords

Bacterial nanocellulose · Surface modified BNC · Metal/BNC composites · Catalysis · BNCs grafted inorganic catalysts · BNC catalyzed chemical reactions · Electrochemical publications of BNC · Environmental remediation

Introduction

Microorganisms are essential for the naturally occurring production of a wide variety of biopolymers, including polysaccharides, polyesters, and polyamides. These biopolymers can take the form of viscous liquids or materials that are similar to plastic. Similar to their petroleum analogues, the physical properties of these materials change with both molecular weight (the degree of polymerization) and composition (the nature of the monomeric units). Biopolymers, also known as cellular polymers, are organic compounds synthesized by cells. The monomeric building blocks of these molecules establish covalent connections to form polymers. Microbial production of custom biopolymers with physico-chemical properties suitable for high-value medical application, including occlusion, suturing, covering, fixing, isolating, adhering, tissue engineering, cellular proliferation, contact inhibition, tissue guide, soft-tissue replacement vascular grafts, and breast implants, has enormous potentials and a completely benign pathway thanks to advances in genetic manipulation of microorganisms with cutting-edge biotechnological methods. Xanthan, alginate, cellulose, cyanophycin, poly(γ -glutamic acid), levan, hyaluronic acid, organic acids,

oligosaccharides, polysaccharides, and polyhydroxyalkanoates are only a few of the many important molecules in this large family.

Biopolymers can be broken down further into subcategories based on the monomers used and the overall structure of the biopolymer; for example, polynucleotides, polypeptides, and polysaccharides. Polynucleotides, such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), are long polymers composed of 13 or more nucleotide monomers. Collagen, actin, and fibrin are just a few examples of the proteins and polypeptides that are made up of chains of amino acids. Starch, cellulose, glycogen, alginate, heparin, and a number of other substances are all examples of polysaccharides, which are linear or branched polymeric carbohydrates. Natural rubber (isoprene polymers), chitin (the second most abundant natural polysaccharide after cellulose), and gamma globulins (N-acetyl-hexosamine, D-mannose, D-galactose), a class of globulins, identifiable by their location after serum protein electrophoresis are also examples of biopolymers. Complex polyphenolic polymers like suberin and lignin, complex polymers of long-chain fatty acids like cutin and cutan, and the pigment melanin, responsible for the coloration of hair, eyes, skin, and other body parts.

Naturally produced molecules/materials from substantially hierarchical structure are promising possibilities for high-performance and functional advanced materials due to their renewability, non-toxicity, biocompatibility, biodegradability, flexibility, increased availability, cost-effectiveness, and eco-friendliness compared to non-biodegradable petroleum-based polymers. Along with these inherent properties, (bio) enabled nanocomposites based on biopolymers and materials from chemical sources can benefit greatly from the synergistic combination of biological and synthetic elements made possible by powerful interfacial interactions or surface modifications, leading to enhanced structural performance and the introduction of novel functions/applications. Consequently, these natural materials have several benefits in comparison to synthetic polymers, especially in the areas of society, the economy, and the environment. In addition, when compared to biopolymers, the use of polymeric materials derived from petroleum has a far more detrimental effect on the long-term viability of the ecosystem.

The term “BNC” was not used publicly for the first time until 1886, when Adrian Brown did so. Studies of the chemical processes of *Bacterium aceti* also revealed the presence of an acetic ferment, or “mother of vinegar,” which is well-known for its ability to create acetic acid. The resulting coating on the medium’s surface was not as permeable as that produced by *Bacterium acetiana* and had some tactile similarities to an animal membrane. As a consequence of testing this film with various chemical solutions, Brown was able to conclude that the “vinegar plant” indeed did exhibit all the properties of cellulose. Bacterial nano-cellulose (BNC), or microbial cellulose, can be manufactured from microbial cultures in the same way as other biopolymers can. The material is novel in its structure and characteristics, making it an intriguing pioneering biomaterial. Advantages over plant-derived cellulose include higher purity (free of lignin, hemicellulose, and pectin) (Reiniati et al. 2017), higher crystallinity, higher elasticity and con-formability, lower density, higher strength, and greater biodegradability and biodegradability than conventional synthetic fibers

(Bäckdahl et al. 2006). Biologically-fabricated nanocrystalline (BNC) cellulose is generated from well-defined carbon sources by biochemical pathways and the self-assembly of secreted cellulose fibrils in the culture medium. Unlike plant celluloses, which contain functional groups like sulfates, acetates, carbonyl, and carboxyl, BNC contains no such contaminants and is also free of other veg-polymers including lignin, hemicelluloses, and pectin. BNCs, which are synthesized from pure cellulose, have micrometer-scale lengths and average diameters of 20–100 nm, and their network architectures are highly stable. The bacterial nanocellulose was produced through communication between various Gram-negative bacteria, including *Rhizobium*, *Xanthococcus*, *Pseudomonas*, *Azotobacter*, *Aerobacter*, and *Alcaligenes* (Table 1). The improved water-holding capacity and hydrophilicity of BNC in contrast to cellulose obtained from plants have made it possible for enhanced and

Table 1 Representation of the various types of bacteria involved in the biotechnological synthesis of BNC from a suitable carbon source

Name of the bacteria	Gram negative/positive	Carbon source/ agro-waste	Yield (g/L)	Reference
<i>Pseudomonas</i>	Gram negative	Date molasses	4.6	(Kazim 2015)
<i>Acetobacteracetisubsp. xylinus</i> ATCC 23770	Gram negative	Konjac powder	–	(Hong and Qiu 2008)
<i>Acetobacterxylinum</i> NBRC 13693	Gram negative	Orange juice	7.2	(Kurosumi et al. 2009)
<i>Acetobacter</i> sp.	Gram negative	Molasses and corn steep liquor	3.12	(Jung et al. 2010)
<i>Komagataeibactersacrofermentans</i>	Gram negative	Crude glycerol	6.4	(Lee et al. 2021)
<i>Gluconacetobactersacchari</i>	Gram negative	Dry olive mill residue	0.85	(Gomes et al. 2013)
<i>Gluconacetobacterxylinus</i>	Gram negative	Waste dyed cotton fabrics	1.3	(Guo et al. 2016)
<i>Gluconacetobacterxylinus</i>	Gram negative	Grape pomace extract	6.7	(Cerrutti et al. 2016)
<i>Gluconacetobactersacrofermentans</i>	Gram negative	Thin Stillage Whey HS medium	6.19 5.45 2.14	(Singhsa et al. 2018)
<i>Komagataeibactermedellinensis</i> ID13488 strain	Gram negative	Grass	6 0.0	(Jeremic et al. 2019)
<i>Medusomycesgisevii</i>	Gram negative	Oat hulls	–	(Sakovich et al. 2017)
<i>Komagataeibactersaccharivorans</i> MD 1 strain	Gram-negative	Palm date fruits, fig fruits, and sugarcane molasses	3.9 g/L	(Abol-Fotouh et al. 2020)

wider applications of nanocellulose, particularly in the fields of biotechnology, medicine, pharmaceuticals, and the food industry.

In the manufacturing of more than 80% of all goods derived from sulfuric acid acetone, catalysis is used at some stage as a technique that is involved in the processing of the product. Therefore, there are three reasons why this physical process is so crucial: (a) It helps the world's largest economies succeed economically. (b) It makes possible the production of things necessary for the upkeep of modern society. Thirdly, it has made chemical production far more eco-friendly and sustainable as a whole. There are four main types of catalysts: (1) the homogeneous catalyst, (2) the heterogeneous (solid) catalyst, (3) the heterogenized homogeneous catalyst, and (4) the biocatalyst. When compared to homogeneous catalysts/heterogenized homogeneous catalyst, heterogeneous catalysts have various benefits in terms of sustainable technology. These include being easy to handle, store, recycle, and separate the catalyst and product. Vanadium oxides, Fe-Mo, Pt-Rh gauze, zeolites, clays, alumina, zirconia, etc. are all examples of commercial catalysts. Nanocelluloses, or cellulose-based nanomaterials, are currently the focus of much research. In particular, the use of nanocellulose as a catalyst support in various catalytic reactions has garnered a lot of attention over the past few decades. Bacterial nanocellulose is a suitable catalyst support because it has superior chemical, colloidal, optical, and mechanical properties compared to other nanocelluloses (CNC, CNF, and BNC). So, there has been a lot of time and effort put into studying and potentially using these sustainable heterogeneous catalysts, but they have not yet been put to use in a commercial setting.

In this chapter, we provide an overview of recent synthetic methods for the production of metal/BNC composites with high metal disparity and large surface areas, and therefore superior catalytic activities. These composites can be used in a variety of applications, including catalysis, chemical synthesis, and fuel cell applications. Synthetic methods that altered the morphological and chemical functionality of BNC in order to improve its physicochemical qualities as a substrate for more effective metal stabilization, which led to an increase in the catalytic activity of the material. After that, the existing organic transformations that can be accelerated by such BNC-based catalysts are shown. In this chapter, a prospective outlook on the topic is discussed, and anticipated future developments in research on BNC-based materials in (nano) catalysis are broken down in depth.

Synthetic Approaches Towards Bacterial Nanocelluloses (BNCs)

The chapter provides a brief overview of the many synthetic methods that have been applied to the production of bacterial cellulose. As a potential carbon source, agro-industrial waste from a variety of sources has been employed in cultivation. Production of BNC from agro-industrial by-products and cellulosic residues has been the subject of previous research. These materials have included konjacglucomannan (Hong and Qiu 2008), wheat straw (Hong et al. 2011), waste fiber sludge (Cavka et al. 2013), waste cotton textile (Hong et al. 2012), and sweet sorghum bagasse

(Wang et al. 2021). Using naphthalene crystals as its only carbon source, the *Xanthobacter* sp. strain *Starkeya* sp. strain N1 was able to produce cellulose nanofibrils (Marín et al. 2019). This process is more socially responsible because it recycles hazardous polycyclic aromatic hydrocarbons like naphthalene. The discovery of a nanocellulose network with a diameter of 33 nm paves the path for a valuable polymer with broad biotechnological applications.

Enzymatic hydrolysis of the oat husks pulp to furnish a solution of reducing sugars, principally glucose, followed by creation of a nutritional broth based on the enzymatic hydrolysate and biosynthesis of nanocellulose microfibrils by the symbiotic *Medusomyces gisevii* Sa-12 culture and purification of BNC employs an appropriate approach for the chemical transformation of the feedstock (Sakovich et al. 2017). Bacterial nanocellulose (BNC) was produced using *Gluconacetobacter xylinus* and waste colored cotton fabrics (Guo et al. 2016). Through static fermentation, the BNC-producing *Komagataeibacter medellinensis* ID13488 strain was able to extract BNC from sugars produced from grass biomass at yields of up to 6 g L⁻¹ (Jeremic et al. 2019). Purified, separated, and mechanically treated Kombucha membrane, produced by fermenting tea broth with a symbiotic culture of bacteria and yeasts, yields bacterial cellulose nanofibrils (Dima et al. 2017). Under static conditions, *Gluconacetobacter xylinus* used grape pomace extract (a wine industry byproduct) as a carbon source and maize steep liquor (a byproduct of corn wet milling) as a nitrogen source to create bacterial nanocellulose (BNC) (Cerrutti et al. 2016). Recently published works explain a comprehensive study on the influence of the BNC production parameters and its use in transition metal nanocomposites for the catalysis of cross-coupling reactions, and provide a detailed discussion on the influence of the single parameters, such as pH, glucose concentration, and day of incubation, for the optimization of BNC production. The treatment of grass biomass yields a sugar combination that can be used as a carbon source for BNC production, leading to the valorization of non-food renewable biomass. As of Thiruvengadam and Vitta (2017).

Carbon nanotubes were added to the synthetic media in a select number among these studies. Structure study performed after 2 weeks of incubation showed that bacterial cellulose fibrils had coiled around the carbon nanotubes (Yan et al. 2008). As a composite synthesis technique, in situ poly-3-hydroxybutyrate was used by Ruka et al. (2013) to alter the shape and crystallinity of bacterial cellulose (Ruka et al. 2013). Serafica et al. (2002) developed bacterial cellulose composites by feeding shake cultures with metals including aluminum and iron. It was observed that the concentration, spin speed, and particle size of a suspension all play a role in how easily the particles in that suspension can pass through a membrane (Serafica et al. 2002). Ex-situ synthesis can occur by either a physical interaction (such as OH-binding) or a chemical interaction (also possible). The matrix of bacterial cellulose is porous, thus it may physically absorb or let through liquids and small solid particles. With this synthesis technique, the polymer structure is kept relatively intact. The production of composites for applications in the biomedical and industrial sectors makes extensive use of nanocelluloses grown in static culture (Maneerung et al. 2008). In addition, a sizeable number of researchers have made

important contributions to the production of a large number of compounds that can be made from polymers, inorganic materials, metals, metal oxides, and bacterial cellulose. These compounds have been manufactured in a laboratory setting (Evans et al. 2003).

Synthesized polymer compounds are in two fundamental forms, in-situ and ex-situ, depending on the nature of the combination agents and the method to composite development, In-situ synthesis and ex-situ synthesis. The in-situ approach uses the addition of material during its synthesis, which then becomes part of the polymer. In the ex situ approach, the polymer matrix is impregnated with reinforcing elements to generate the composites (Saibuatong and Phisalaphong 2010). In addition, many researchers have worked to perfect and perfect new compounds made from polymers, inorganic materials, metals, metal oxides, and even bacterial cellulose for use in a wide range of fields.

Properties and Surface Modification of BNCs

The building blocks of cellulose are D-glucose monomers linked together by β -1, 4-C-O-C- glycosidic bonds (Ross et al. 1991). The most important characteristics of bacterial cellulose are its high levels of mechanical strength, purity, crystallinity, and water content. Bacterial cellulose also has a high degree of crystallinity (Klemm et al. 2005). Structure that is thin, extremely hydrophilic, and porous that is produced from bacterial cellulose microfibrils that are arranged in a nanofibrillar network in three dimensions. Because of its porous structure, nanobacterial cellulose is useful in many contexts, whether it is wet or dry.

Its resistance to enzymatic and chemical hydrolysis is due to the fact that bacterial cellulose is insoluble in water and other common organic solvents. Additionally, its supramolecular organization, which stabilizes the glucan chains via inter-chain and intra-chain hydrogen bonds, also contributes to its resistance. Because of its linear cellulose chains and strong cohesion between macromolecules, bacterial cellulose exhibits remarkable mechanical strength (Klemm et al. 2006). Young's modulus of bacterial cellulose fibers ranges from 78 GPa (Guhados et al. 2005) to 114 GPa (Raman spectroscopy) (Hsieh et al. 2008). There is no difference in terms of their mechanical properties between filaments of bacterial cellulose that vary in diameter (Guhados et al. 2005). When bacterial cellulose membranes are exposed to air, the Young's modulus increases to around 9.7 GPa, the stress and strain at break increases to over 240 MPa, and the strain at break increases to around 2.6%. Rheological investigations of bacterial nanocellulose fibers exhibit stress-softening behavior due to the destruction of physical cross-links and bending of fibers, followed by a rearrangement of the network due to the synthesis of new cross-links. The loss modulus also decreases after a specific stress level is attained. Bacterial cellulose can have a crystallinity level of around 80%; however, this varies greatly depending on factors like the composition of the medium and whether or not the product is formed in a static or agitated environment during cultivation (Jung et al. 2010; Trovatti et al. 2011).

Inorganic Functionalization of BNCs and Catalytic Applications

Numerous inorganic substances, including as metal oxides, metal sulfides, silica, or metal nanoparticles, have been integrated into BNC by taking advantage of its isotropic three-dimensional nanostructure in order to generate flexible heterogeneous catalysts. These catalysts can be used in a variety of different reactions. Synthetic methods can be used to incorporate these inorganic components into nanocomposites for the purpose of boosting their activity.

Reduction using an external reducing agent is most common approach is to use an external reducing agent to reduce a metal precursor into metal NP on the nanocellulose surface. For example, AgNPs have been synthesized on various types of nanocelluloses by reducing AgNO_3 with NaBH_4 (Ramaraju et al. 2015). Other methods, which have been utilized the most and have been reported in the scientific literature: (i) the mixing under conditions of simple agitation or ultrasound-assisted conditions; (ii) the absorption through solvothermal processes; and (iii) the in situ incorporation in the growth medium. The subsequent sections categorize and discuss the catalytic applications of these novel materials.

Immersing the bacterial nanoellulose in this metal salt solution allowed for the imbuing of metal nanoparticles, which led to the desired catalytic, energy storage, food packing, antibacterial, and piezoelectric activity features. Through a reduction process involving sodium borohydride or ascorbic acid, metal nanoparticles were incorporated into bacterial cellulose. The silver ions were found to be released immediately from the freeze-dried silver nanoparticle-impregnated BC produced with NaBH_4 : AgNO_3 at a molar ratio of 1:1 (silver nanoparticles impregnated on the surface) and gradually when the molar ratio was increased to 10:1 and 100: 1 (deeply impregnate) (deeply impregnate). The freeze-dried BC loaded with silver nanoparticles was effective against both Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus* (Maneerung et al. 2008).

In 2017, Chen et al. used the 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO)- mediated oxidation reaction to take advantage of this capability in order to get a BNC with carboxyl groups on the glucose moieties (TOBNC), which have been subsequently used as a support for gold nanoparticles (Au-TOBNC). This was accomplished in order to obtain a BNC with carboxyl groups on the glucose moieties in Chen et al. (2017). Mild aqueous conditions were used to initiate the oxidation reaction, which was subsequently carried out on slurry of fibrillated BNC treated with TEMPO, NaBr, and NaClO. In the presence of sodium borohydride, TOBNC was mixed with a HAuCl_4 solution to create the Au-TOBNC catalyst. Particle size distribution is more uniform for AuNP-TOBNC than for unsupported AuNPs because the carboxyl groups, in their dissociation form, are able to immobilize the AuNP more tightly. The effect of pH on the number of AuNPs that might be loaded onto a particle has been studied. When the pH was adjusted to a range between 3.2 and 11.3, researchers saw a shift in the suspension's hue that corresponded with the observable increase in AuNPs concentration. There is a good chance that the dissociated or associate form of the carboxyl groups is to blame (Sen et al. 2013). Copper nanoparticles (CuNPs) loaded bacterial cellulose

membranes were fabricated by using an in situ chemical reduction method, which gave sustained release of copper ions. This may contribute to the long-term antibacterial activity and with controlled Cu concentration, and most importantly, the membranes were not cytotoxic to normal human dermal fibroblasts, which allowed them to be used as wound dressings (He et al. 2018). After being soaked in CuCl₂ solutions, BC/Cu membranes were reduced with NaBH₄, resulting in excellent long-term antibacterial activities against both Gram-positive and Gram-negative bacteria (*S. aureus* and *E. coli*). Antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was demonstrated by ZnO-deposited bacterial cellulose composites in the absence of a photocatalytic reaction, suggesting their potential use as a wound dressing and water disinfectant (Janpetch et al. 2016). An amidation reaction between carboxylated bacterial cellulose and dopamine was used to modify the bacterial cellulose that was isolated from *Gluconacetobacter xylinus* (MTCC7795). This reaction was then used to prepare BC-DOPA/rGO/Ag NPs, a composite film that demonstrates antimicrobial activity against Gram-positive (*Staphylococcus aureus* and *Lysinibacillus fusiformis*) and Gram-negative bacterial strains (Khamrai et al. 2019). By fusing dioxyphenyl amine (DOPA) onto bacterial cellulose, a green reduction of Ag⁺ and GO led to the fabrication of a composite transdermal patch containing AgNPs/ rGO. Accelerating wound healing, Ag NPs have been demonstrated to do so by stimulating the growth and migration of fibroblast cells (such as those found in NIH 3T3) and lung epithelial cells (such as those found in A549).

The excellent biocompatibility and bioactivity of hydroxyapatite-bacterial nanocellulose composite materials makes them suitable for use in medicine (Silva-Holguín and Reyes-López 2020). This is made possible by the antibacterial properties of silver nanoparticles. With a turbidimetry-determined 52.99% inhibition of *Pseudomonas aeruginosa* at a 5mM AgNPs concentration, HAp-AgNPs dust was found to have a potent antibacterial effect. As a result of the thinner peptidoglycan layer (20–80 nm) of Gram positive bacteria, AgNPs typically show greater antibacterial activity against Gram negative bacteria (10 nm thick cell wall). Therefore, HAp-AgNPs may be useful in a variety of medical settings. To enhance the colloidal stability of HA nanoparticles, an in situ BNC/hydroxyapatite/cellulose nanocrystal (BHC) composite was synthesized with the aid of CNCs (Niamsap et al. 2019). BHC composite had no cytotoxic potential, as evidenced by the fact that cell viability was 99.20.08% in the negative control and 83.43.6% in cells treated with BHC-0.5 wt% HA and HC (Hydroxyapatite/ cellulose nanocrystals).

The strength-to-weight ratio of BC sponges cross-linked with glucose and citric acid was 150% and 120% higher compared to that of unmodified BC sponge, and such modified BC sponges are non-cytotoxic and do not trigger an inflammatory response in macrophages. Highly porous 3D structures with multi-hierarchical organization were created using bacterial cellulose (Frone et al. 2010). In cases of retinal degeneration, a novel substrate was developed using bacterial cellulose (Gonçalves et al. 2015). Increasing cell-material interactions, RPE cells adhere to and grow on BC-modified surfaces. The hydrophilicity of BC's surface was reduced through acetylation and polysaccharide adsorption using chitosan or carboxymethyl cellulose, while amine or carboxymethyl groups were incorporated into BC via

protein adsorption. Acetylation of BC reduced swelling and the amount of endotoxins and decreased elastic modulus due to the moderately hydrophilic surface after acetylation, but these surface-modified BC substrates exhibited similar permeation coefficients with solutes, stress-strain behavior, and enhancement of adhesion and proliferation of RPE cells.

The bacteria *Gluconacetobacter xylinus* create the bacterial nanocellulose, and a precise and carefully developed multi-step procedure is used to fabricate the magnetic MBNC material (BNC). To sum it up, BNC pellicles are made out of frozen *G. xylinus* strain to meet the specific size and morphological needs of our experiments. Preparing a 2:1 molar ratio solution of iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) and iron (II) chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$) in deoxygenated high purity water. After the reactants have been placed in the vessel, a BNC pellicle is added. For the purpose of precipitating the ferrous ions into the BNC mesh, this mixture is heated to 80°C in a silicon oil bath while being agitated, and then ammonium hydroxide (14% by weight) is dropped into the pot (Fig. 1). This final step allows for the formation of in situ magnetite nanoparticles (Fe_3O_4) inside the bacterial nanocellulose mesh in order to confer magnetic characteristics onto the BNC pellicle. The biocompatibility of the BNC-IONP (iron oxide nanoparticles) pellicle was determined using a toxicological experiment. To increase their biocompatibility, IONPs were coated with polyethylene glycol (PEG). The magnetic properties of the MBNC pellicle were confirmed by magnetic force microscope (MFM) measurements, which revealed the presence of magnetic domains with both strong

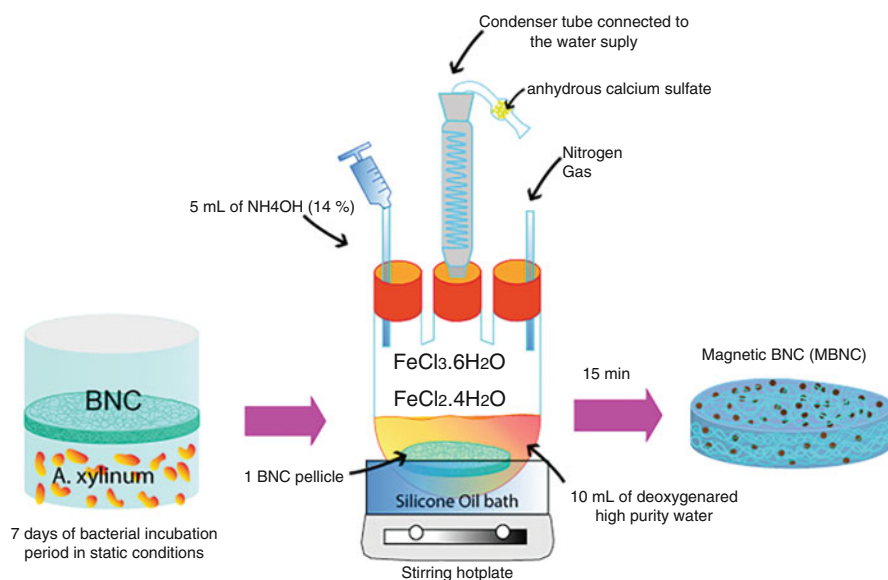


Fig. 1 Schematic representation of the fabrication of magnetically functionalized bacterial nanocellulose. Iron oxide nanoparticles (IONPs) are assembled and incorporated in situ within the BNC, yielding a MBNC. (Adopted from Arias et al. (2016). Copyright@ MyJove Corp; 2022)

and weak intensity magnetic field. The results of this work on Young's modulus are similarly consistent with those reported for a variety of blood arteries in other investigations (Arias et al. 2016).

In this study, we created novel BNC one-dimensional hybrid nanofibers by combining hydrated bacterial cellulose nanofibers with Cu-Pd NPs (Pd = 3.7; Cu = 4.0). To make palladium-copper nanoparticles, potassium borohydride was used to reduce metallic ions absorbed by the BCF during immersion in a solution of palladium chloride (PdCl₂) and copper chloride (CuCl₂) in water. Pd-Cu nanoparticles precipitated uniformly on the BCF surface, as shown by physicochemical characterization of mixed metal/BNC nanocomposites (Sun et al. 2010a).

Very recently, the research group of Kamal et al. proposed several different examples in which BNC was used as a high surface area support for transition metal nanoparticles that were stabilized with carboxymethyl cellulose (CMC), such as cobalt (CMC-Co-BNC) (Kamal et al. 2019a), copper (CMC-Cu-BNC) (Kamal et al. 2019) and nickel (CMC-Ni-BNC) (Kamal et al. 2019b). In these works, a mixture of metal chloride and CMC (1 wt%) solutions was combined, and then hydrazine hydrate and ascorbic acid were added in a slow, controlled fashion. The resultant solution was heated in a microwave, which resulted in the formation of carboxymethyl cellulose-metal nanoparticles (also known as CMC-M). Last but not least, the CMC-M-BNC dip-catalyst was made by spreading a CMC-M suspension on BNC sheets obtained from a *Gluconacetobacterxylinum* culture.

The comparatively low cost of manufacture of ZnO-NPs, antibacterial characteristics, chemical stability, reactive oxygen species (ROS), and photo-catalytic abilities are all factors that contribute to the great features that these nanoparticles exhibit. Zinc oxides were said to be widely employed in the paper, paint, and plastics sectors due to their generally equal absorption of ultraviolet (UV) and visible light (Wang et al. 2019). Excellent catalytic performance, high antibacterial activity, and improved electrical conductivity were observed in a composite of nanocellulose and zinc oxide NPs, and the NPs were also reported to improve the UV-barrier properties, thermal stability values, and antibacterial activities of biopolymer films (Farooq et al. 2020).

Catalytic Transformation of Organic Compounds for Environmental Purposes

Numerous attempts have been made in order to make use of the one-of-a-kind features that bacterial nanocellulose possesses, such as its bioavailability and aqueous solubility, which brings in some very interesting advantages towards the application of bacterial nanocellulose in catalysis. These additions have been brought about as a result of the numerous efforts that have been made. Recent research has also shown how these state-of-the-art compounds perform as catalysts for reactions like electrocatalysis, reduction, oxidation, and coupling. In this case, BNCs can serve as a platform or support material for the active metal catalyst, helping to keep it stable. The catalytic transformation of organic compounds is an area of active

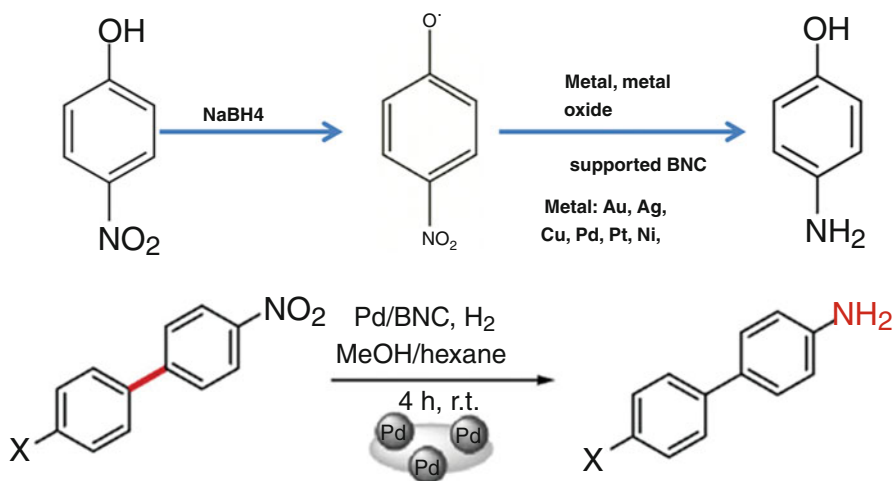
research, and nanoscale cellulose materials functionalized with metallic systems are of particular interest. Renewable natural resources, such as microbial cellulose, can be used to create these goods. Conventional uses for CNC-based materials include reinforcement in the plastics and paper industries, but these materials have also been put to work in a wide range of cutting-edge applications for environmental remediation, including as adsorbents, catalysts, photocatalysts, flocculants, and membranes. Such applications are typical. Catalysts based on BNCs are highly active and selective for a wide range of organic transformations, while retaining a key advantage of heterogeneous catalysis – the ability to separate products with relative simplicity.

Reduction Reactions

In commercial settings, the reduction reaction is typically carried out in a blast furnace. A blast furnace is a specific kind of furnace that is utilized during the smelting process that is necessary for the production of iron. The method of making iron involves chemically reducing iron oxide to a liquid state. The zinc case of a dry cell serves as the anode, and the reduction of MnO_2 takes place at this site; this reaction has various applications in industry, including those involving batteries and cells.

The conversion of 4-nitrophenol to 4-aminophenol has seen extensive use of hybrid nano-composites comprised of metal nanoparticles and nanocellulose. Tests of this reaction have been performed on a variety of other NPs, including gold (Au), silver (Ag), platinum (Pt), and gold (Au-Pd) NPs. Metal NPs supported on nanocelluloses undergo a reduction reaction in the presence of NaBH_4 at room temperature, with the reaction being completed between 15 and 30 min and yielding excellent conversions of 95–100%. Metal NPs supported on nanocelluloses reduced 4-nitrophenol to 4-aminophenol at various turnover frequencies (TOFs). An Au-TOBNC catalyst was tested to see if it could reduce 4-nitrophenol (4-NP) using NaBH_4 . 4-NP was used as a model reaction. Support from TOBNC increases the rate at which 4-nitrophenol (2-NP) is converted to 4-aminophenol (6-AP) by about 20-fold compared to AuNP alone. Since the TOBNC increased the amount of reagent exposed to each nanofiber, it is likely that the Au reagent was more evenly distributed. After examining how different temperatures affected the reduction reaction, scientists settled on 65 °C as the sweet spot for maximizing the efficiency of both the substrate and the products. There has been no other work that has produced such a high level of catalytic efficiency for an analogous catalyst (Sen et al. 2013).

The nitro group in biphenyl-4-amine (4) and 4'-fluorobiphenyl-4-amine (5) was efficiently reduced using Pd/BNC in the corresponding process. Pd/BNC was typically added to the chemical in ethanol and hexane before the reaction mixture was purged with hydrogen. Thus formed amalgam was swirled at constant rate in a 3 h long hydrogen-filled environment. After filtering the reaction mixture and evaporating the solvent at low pressure, 4.32 mg (99%) of a white solid was



Scheme 1 Reduction reactions by metal NPs-(bacterial) nanocellulose hybrid composites, the reduction of 4-nitrophenol to 4-aminophenol (top) and para-nitro-biphenyl derivative in to para-amino biphenyl (b), which is considered as a model reaction in heterogeneous catalytic reactions

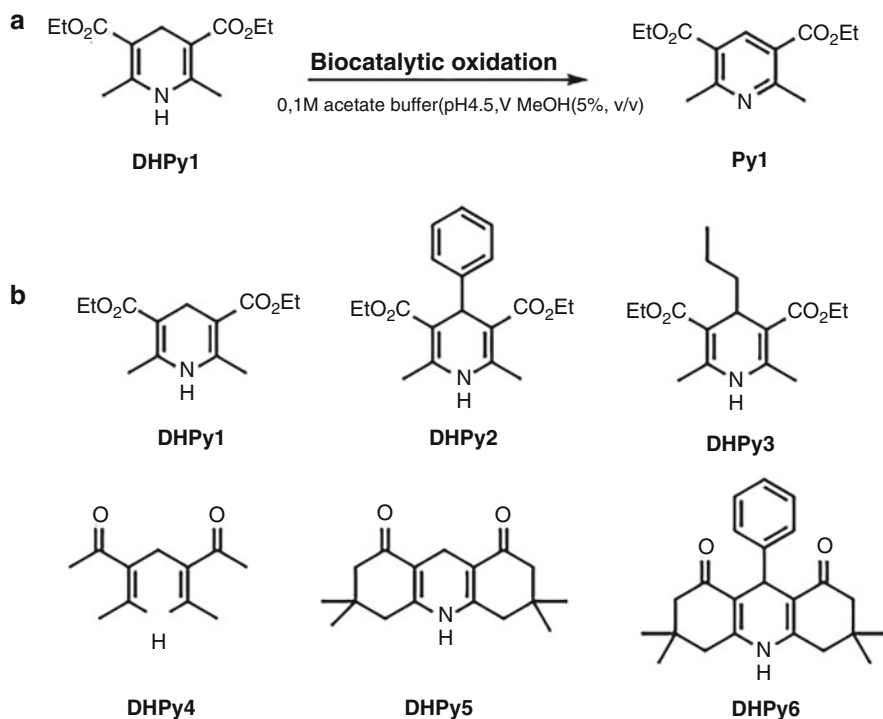
obtained. Pd/BNC catalyst has been used for nitro group reduction to amino group hydride reduction (Scheme 1). It is important to note that quantifiable yields of amines were produced even though no additional purification procedures were utilized. When the eco-friendly catalyst was used, it was easily recovered and put to use in two separate reactions of a different type.

Oxidation Reactions

Oxidation reactions are not only extremely important in the natural world, but they also play a significant role in the process of organic synthesis as critical transformations. Utilizing oxidizers such as molecular oxygen, hydrogen peroxide, and osmium tetroxide, the chemical industry makes substantial use of oxidation reactions. Due to the ease with which excess gas may be evacuated from the reaction, the development of greener processes is facilitated when traceless gaseous reagents are used in place of chemicals. Organic synthesis is mostly dependent on oxidation processes, which modify or add functional groups to molecules. Chemoselectivity and regioselectivity, on the other hand, are frequently low in autooxidations and other processes involving radical intermediates. Large amounts of waste are produced when stoichiometric metal oxidants are used, such as KMnO_4 or K_2CrO_4 , and they are not always environmentally sustainable.

Biocatalytic oxidations that are mediated by laccases are becoming increasingly significant as a result of their adaptability as well as the good impacts that they have on the environment that they are surrounded by. Several bacterial laccase-based

catalysts have been employed for the oxidation of 1,4-dihydropyridines. These are BNC supported BliLacc catalysts, whole *Escherichia coli* cells expressing this laccase, and pure laccase from *Bacillus licheniformis* ATCC 9945a (BliLacc). Catalysts based on bacterial laccase were compared to the commercially available *Trametes versicolor* laccase (TvLacc). Good yields (70–99%) of the 2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate oxidation product were achieved with all three biocatalysts in as little as 7–24 h. Regarding substrate diversity, five more 1,4-dihydropyridines were investigated (Scheme 2). Whole-cell biocatalysts were shown to be relatively stable for up to one month of storage at 4 °C. And there's more evidence that the *E. coli* multicopperoxidase CuO is effective. It has been demonstrated that the *E. coli* expression host enhanced the whole-cell biocatalyst's oxidation efficiency. Reusing previously used *E. coli* (BliLacc) could grow on BNC, however the reaction yield dropped nearly linearly after three rounds of growth. In addition, after three biotransformation cycles, the immobilized whole-cell biocatalyst retained 37% of its initial activity. Several of the biocatalytic systems



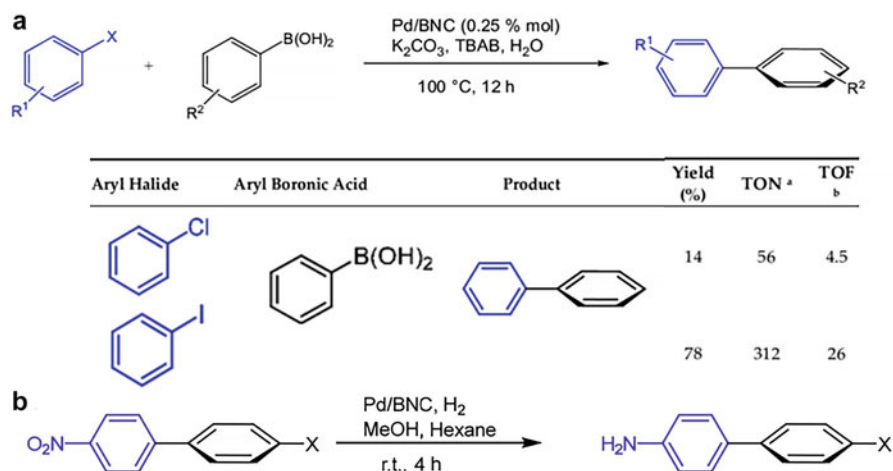
Scheme 2 Schematic representation of the chemical structure and the biocatalytic oxidation of Hantzsch 1,4-dihydropyridines by employing biocatalysts based on bacterial laccase. Diethyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (DHPy1) was converted to diethyl-2,6-dimethylpyridine-3,5-dicarboxylate (Py1) and 1,4-dihydropyridine (DHPy2–DHPy6) substrates for this work (b). Except for DHPy6, all substrates produced the matching pyridines

developed in this study can be applied to additional laccase-mediated processes (Simić et al. 2020).

Coupling Reactions

There is a high demand for heterogeneous catalysts to be used in organic synthesis as well, and BNC meets this need thanks to its unique properties, such as its high functionalization selectivity. There is a wide variety of applications for BNC, but one of the most common is as a support for the synthesis of heterogeneous catalysts through the use of straightforward metal loading on the BNC surface. Metals such as Pt(II), Pd(II), or Cu(I), Cu(II) salts in sodium borohydride solution in water under mild reaction conditions are used to load onto BNC. Surface metal dispersion on catalysts has been found to vary, indicating varying degrees of affinity with the BNC. Using quadrupole inductively coupled plasma mass spectrometry (ICP-QMS), researchers found that the amount of Cu on Cu/BNC was tenfold lower than Pd on Pd/BNC, indicating more efficient incorporation for the Pd catalyst than the Cu catalyst. Also, Cu formed spheres on the surface, while Pd nanoparticles were embedded in the BNC structure. The eco-friendliness of the Pd/BNC grafted catalyst is highlighted by its use as catalysts for the Suzuki-Miyaura reaction in mild reaction conditions with water as the solvent. Using bromobenzene with various arylboronic acids resulted in increased yields during the coupling reaction (Scheme 3a).

Pd/BNC was also utilized in the process of obtaining 4-methoxybiphenyl in water using a method that did not involve the use of ligands. A colorless solid was obtained

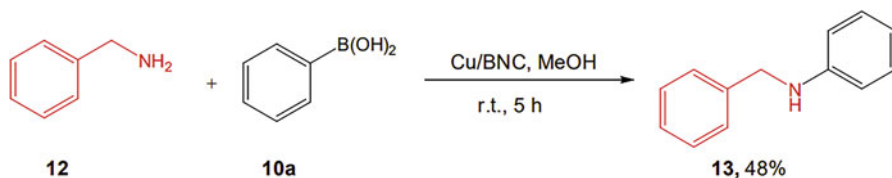


Scheme 3 Pd/BNC catalyzed Suzuki–Miyaura reaction of aryl halogenides with arylboronic acid in water, where TON: Turnover number and TOF: Turnover frequency (**a**), reduction of nitro-group catalyzed by Pd/BNC catalyst, which demonstrates the potential of the catalyst towards reuse under the experimental conditions (**b**), where X can be H or F

as a byproduct of the standard Suzuki reaction protocol. To obtain 4-nitrobiphenyl without the use of ligands, Pd/BNC was also used (Zhang 2005). In accordance with the procedure that is considered standard operating procedure for the Suzuki reaction, the compound was successfully obtained as a solid yellow substance. 4-Fluoro-4'-nitrobiphenyl: Using ligand-free methodology, Pd/BNC was also used to obtain 4-fluoro-4'-nitrobiphenyl in water (Scheme 1) (Samarasimhareddy et al. 2013). In accordance with the standard operating procedure for the Suzuki reaction, a yellow solid form of the compound comprising approximately 91% was obtained. Throughout the course of the catalytic recycling experiments, they have used inductively coupled plasma (ICP) emission spectroscopy to examine the products and found that there was no Pd contamination to be found in any of them. SEM analysis further confirms the stable morphology during recycling of Pd/BNC catalyst after 4 reaction cycles.

Both from an economic and an environmental point of view, the recovery and reuse of a spent catalyst is important. Both of these factors contribute to the importance of this practice. After a catalyst has served its purpose, it is considered “spent,” and poses a danger to the environment because of the metals that can be leached from its surface. Consequently, a catalyst’s usefulness depends largely on its resistance to extreme reaction conditions and its potential for recycling. The reduced nitro to amino group using hydrogen gas was catalyzed by the recovered catalyst with a 99% yield for both compounds (Scheme 3b). With a yield of 99%, the ability of the BNC-based catalyst to be reused is demonstrated in a clear and convincing manner.

When an aryl boronic acid is mixed with either an alcohol or an amine, a sort of cross-coupling reaction known as the Chan–Evans–Lam coupling takes place. This reaction is a type of cross-coupling reaction. Secondary aryl amine or aryl ether is formed during this process, depending on the molecule being linked. However, using copper complexes to catalyze the reaction raises concerns about the process’s repeatability and, by extension, its environmental sustainability. The Cu/BNC nanocatalyst has been employed as a heterogeneous catalyst for the Chan–Lam coupling reaction between benzylamine and phenylboronic acid (Scheme 4). The authors speculated that low Cu incorporation on BNC may have caused the modest output. The effectiveness of these materials as heterogeneous catalysts was, however, shown by the low amount of metal leaching and the straightforward recovery techniques.



Scheme 4 BNC grafted with copper nanoparticles as a sustainable green catalyst for the coupling of benzylamine and phenylboronic acid

Electrocatalytic Applications

Ozone has a wide variety of applications that go well beyond the realm of environmental science. One of the most powerful oxidants is O_3 , which has an oxidation potential of 2.07 V. Because of its potent oxidative property, ozone is an effective chemical for sanitizing and disinfecting environments (Beltrán 2004; Cheremisinoff 2001). In the process of disinfecting water systems, microorganisms are eradicated, organic molecules are decomposed, and cyanide, phenols, manganese, detergents, and colors are removed. It is used to purify drinking water, food, and surgical tools, in addition to the purification of wastewater and the water in swimming pools. This is also brought to use in a broad variety of other contexts, such as those listed above. It is a raw ingredient in the production of several organic compounds like oleic acid and peroxyacetic acid. Ozone is a great option for water purification because it does not leave behind any toxic byproducts. (Han et al. 2004). However, ozone is extremely volatile, with a half-life of only 30 min in water. Therefore, it is widely employed by technologies for production in situ. In comparison to other methods of production, electrocatalytic ozone generation technology is viewed as a more environmentally friendly chemical process. In-situ sterilization and decolourization applications can benefit greatly from eco-friendly electrochemical ozone production (EOP). Ozone can be synthesized electrochemically using a nanocatalyst composed of Pt single atoms (SAs) embedded in BNC nanotubes (Pt-Sas/BNC). The enhanced performance of the EOP in neutral media can be attributed to the process, which creates a local environment abundant in oxygen intermediates and thus modifies the EOP's thermodynamics and kinetics. At a current density of 50 mA cm^{-2} , the Pt-SAs/BNC catalyst displays excellent EOP performance, with a Faraday efficiency of 21%, nearly double that of commercial $-PbO_2$. The significant performance of the Pt-SAs/BNC nanocatalyst material in electrodegradation of various pesticides and antibiotics is further evidence of the potential environmental application capabilities of Pt-SAs/BNC, in addition to its use in O_3 (Gu et al. 2021).

The utilization of environmentally friendly components in the production of renewable energy technologies that are predicated on the production of electrode materials that are based on BNC for the purpose of electrocatalytic hydrogen production that is both effective and efficient. Conductive flexible substrates made from BNC isolated from *Komagataeibactersucrofermentans* were used in electrocatalytic experiments. Electrodeposition in a Ni and Mo solution (Fig. 2) transformed the conductive BNC substrate into an active electrode for the hydrogen evolution reaction (HER) under basic conditions. Since the BNC electrode is binder-free and has a highly nano-porous structure, the surface active sites are amplified, and the electrolyte shows remarkable catalytic hydrogen production under basic conditions. According to the results of the electrochemical studies, the $NiMoO_4$ /BNC electrode needs an overpotential of 109 mV with a Tafel slope of 170 mV dec^{-1} in 1 M KOH to produce a current density of 10 mA cm^2 . And after 48 h of electrolysis in an alkaline medium, the electrode stands up well (Abraham et al. 2020).

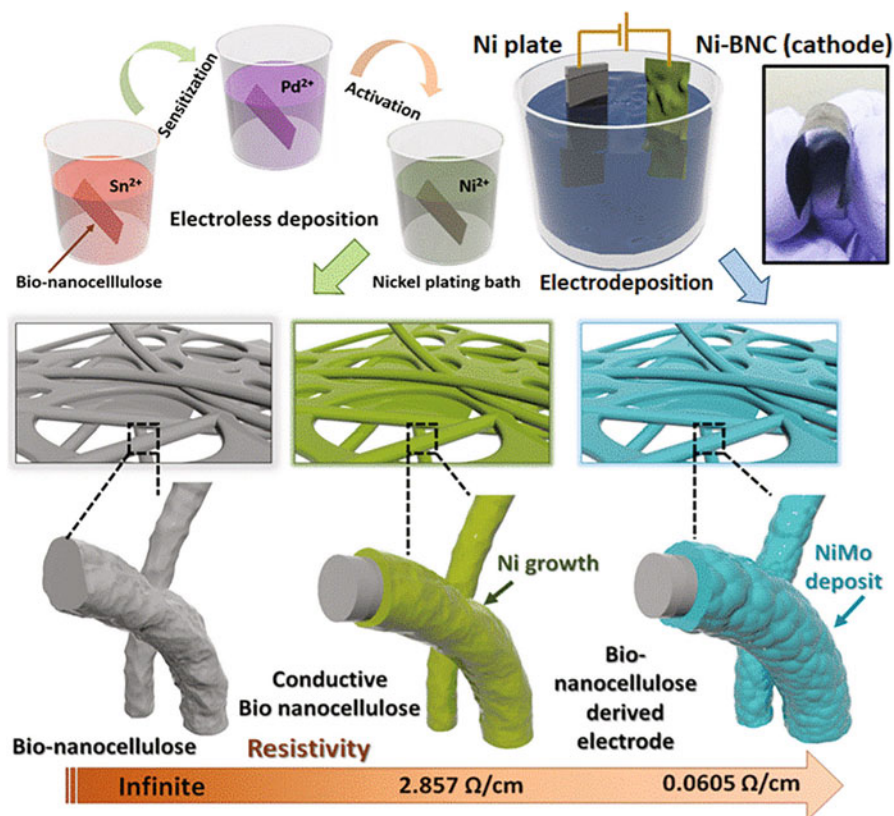


Fig. 2 A scheme showing the fabrication of flexible Ni-Mo/BNC electrocatalytic electrode from *Komagataeibactersufofermentans* derived BNC for hydrogen evolution reaction. (Inspired by Abraham et al. 2020. Copyright © 2022 Elsevier B.V)

By employing an in-situ chemical reduction method, a membrane made of BC that supports CuO/Cu nanocomposites and is relatively inexpensive to produce was created. Electroactive nanocomposite electrodes were grown in situ using CuO/Cu with bacterial cellulose as support. The electrocatalyst (CuO/Cu₄:3@BC) has been used successfully for the electroreduction of CO₂ to CO. As prepared, the CuO/Cu₄:3@BC electrode exhibits superior charge transport property and higher current density for CO₂ reduction in comparison to carbon paper-supported CuO/Cu composite catalysts. This is because the web-like porous structure of the BC substrate provides easier access to active sites to electrolytes and faster transfer of reactants and products. Therefore, the CuO/Cu₄:3@BC electrode's architecturally arranged CuO/Cu composite catalysts offer unique structural advantages of good ion and charge transport, thereby promoting the catalytic kinetics for the conversion of CO₂ to CO. The resulting electrode is capable of producing CO with a Faradaic efficiency (which is also called *coulombic efficiency* or *current efficiency* describes the

efficiency with which charge (electrons) is transferred in a system facilitating an electrochemical reaction) of 53% at a modest over-potential of 490 mV, and it maintains its activity for a period of over 40 h (Zhou et al. 2020). On a platform that is very similar to this one, Gao and his colleagues have reported that a CNT/Bacterial Cellulose (BC) nanocomposite with conductive properties and a large surface area was in-situ cultivated in order to preserve the native 3D nanofibril network. Electrodes effective at reducing carbon dioxide (CO_2) were fabricated in situ using a Cu-Cu_xO/CNT/BC nanocomposite electrode as a support and either potassium dihydrogen phosphate (KBH_4) or sodium hydrogen phosphate (NaBH_4) as a reducing agent (ECR- CO_2). For the purpose of converting ECR- CO_2 into CO and C_2H_4 (Fig. 3), the performance of both electrodes was superior to that of the conventional carbon cloth or pure BC supported Cu-based catalysts. The current density in the double layer increased to 33.3 mA cm^{-2} , and the capacitance increased to 11.8 mF cm^{-2} . The Faraday efficiency ($\text{FECO} + \text{FEC}_2\text{H}_4$) was 66% at a potential of 1.5 VRHE. The Cu-Cu_xO/CNT/BC nanocomposite electrode also showed remarkable stability, remaining functional in ECR- CO_2 for over 20 h (Gao et al. 2022).

Silva and colleagues have reported on the development of skin-adherent biosensors that are based on the use of pure microbial nanocellulose fibers as the substrate (Silva et al. 2020). Screen-printed carbon electrodes (SPCEs) on bare-nylon-coated (BNC) substrates have been reported as a flexible strategy for engineering wearable sensing platforms, as they integrate well with human skin. The use of SPCEs on MNC makes it possible to determine whether or not sweat contains uric acid, 17-estradiol, Pb^{2+} , or Cd^{2+} . The as-prepared SPCEs were able to detect the toxic metals cadmium (Cd^{2+}) and lead (Pb^{2+}) in human sweat and urine at limits of detection of 1.01 and 0.43 M, respectively. The detection limits for uric acid and 17-estradiol in synthetic sweat were 1.8 M and 0.58 M, respectively, when using SPCEs functionalized via anodic pre-treatments. The electrochemical treatment increased wettability and hydrophilicity by adding oxygen groups to the carbon

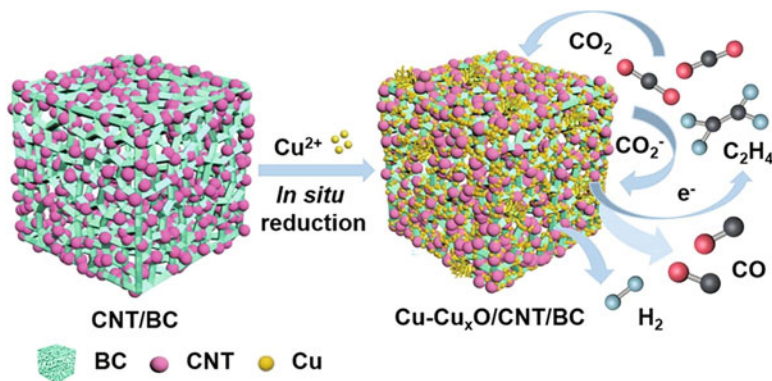


Fig. 3 A simple scheme showing the Cu-Cu_xO/CNT/BC nanocomposite electrode for converting ECR- CO_2 into CO and C_2H_4 . (Inspired by, Gao et al. 2022. Copyright © 2022 Elsevier B.V)

surfaces. In this work, BNC was used as an adhesive-free, yet highly skin-adherent platform for wearable sensing devices, taking advantage of BNC's semi-permeable, non-allergenic, and renewable properties, which set it apart from other materials that have been used for the whole purpose. These findings may pave the way for combining immunosensing devices and have clear ramifications for the development of greener, more biocompatible, and more efficient substrates. Moreover, these findings may pave the way for the combination of immunosensing devices (Silva et al. 2020).

Studies have been conducted on the in situ deposition of platinum (Pt) nanoparticles on bacterial nanocellulose membranes (BNC) for the application of fuel cells. The results of morphological (TEM) and crystallographic (XRD) examinations reveal that the BNC fibrils are well impregnated with spherical metallic platinum nanoparticles with a mean diameter of 3–4 nm. The Pt/BC composite materials have extremely high thermal stability, according to TG's thermal analysis. CV looked into the electrosorption of hydrogen (cyclic voltammetry). The electrocatalytic activity of Pt/BC catalysts in the hydrogen oxidation reaction was found to be quite high. Under non-humidified conditions, the single cell performance of Pt/BC was tested at 20 °C, 30 °C, and 40 °C. Renewable BC is a promising material to be investigated as a membrane in the field of fuel cells, according to preliminary testing on a single cell (Evans et al. 2003; Yang et al. 2009).

Other Types of Catalytic Transformation

The membranes made of bacterial nanocellulose/spiropyran nanofibrous have a photochromic effect (Hu et al., 2011). When exposed to ultraviolet light (UV), BNC/spiropyran photochromic membranes turn pink (merocyanine structure), but when exposed to visible light (spiropyran structure), they revert to their original color (colorless). In a comparable pattern, Gutierrez et al. demonstrated the fabrication of photochromic vanadium nanopapers by using BNC templates and the sol-gel process. Polyoxometalates, also known as POMs, are complex molecules that are composed of highly symmetrical metal clusters that are formed by a number of different metallic ions that are coordinated by shared oxide ions. Because of their structures and properties, they could be used as functional molecules in organic-inorganic matrices. Due to the formation of mixed valence cations, the metallic ions in the POM structure are able to undergo reversible redox process, resulting in the appearance of colored species (heteropolyblues and heteropolybrowns). In photo and electrochromic applications, its redox property and high electron density are highly desirable. Phosphotungstic acid (PWA), with the molecular formula $\text{H}_3\text{PW}_{12}\text{O}_{40}$, is one of the most significant POM that has been investigated in the preparation of new OINs due to its good solubility in water-based and polar solvents. Additionally, the structure of size Keggin has been defined by PWA, where the photoreduction of its W^{6+} sites in response to ultraviolet irradiation is responsible for the majority of the color change. The color shift is due to the photoreduction of $\text{H}_3\text{PW}_{12}\text{O}_{40}$, which is triggered by ultraviolet light. At room temperature, Santos

and colleagues soaked H3PW12O40 in BNC membrane mats made from the *Gluconacetobacter xylinus* (ATCC 23760) bacterium to create photochromic organic-inorganic nanocomposites. It was possible to create a paper-like, semitransparent BNC/PWA nanocomposite. Spectral studies showed that the primary Keggin structure was maintained in the nanocomposites, and exposing a colorless BNC/PWA nanocomposite to UV light caused a change from a bluish semitransparent color sheet to a bluish opaque one. The degree of blueness is proportional to the amount of ultraviolet radiation, and the color returned to normal after the nanocomposites were exposed to air (Santos et al. 2021).

(Photo)Catalytic Transformation of Organics for Environmental Remediation

In recent years, a significant amount of work has been made into the treatment of wastewater coming from industrial sources, with the intention of lowering or getting rid of pollutant levels. Azoic dye usage in the textile sector is a significant contributor to water contamination. Since transition metal-based nanoparticles are less expensive to manufacture than noble metal nanoparticles, we investigated their potential to degrade these pollutants. Thiruvengadam and Vitta (2017) advocated the use of BNC as a flexible and multifunctional Ni-based nanocomposite. They did this by employing methyl orange (MO) as a model dye pollutant (Thiruvengadam and Vitta 2017). The authors generated Nickel-Bacterial Nanocellulose (NiBNC) nanocomposites with varied amounts of nickel by employing a method referred to as “inverse chemical reduction.” Method: Sodium borohydride (NaBH_4) and nickel chloride (NiCl_2) solutions were applied to a BNC hydrogel (NiCl_2). After being hot-pressed at 70 °C and 4 megapascals, the resulting black NiBNC sheet formed a BNC network of strongly linked metal nanoparticles that displayed magnetic and electrical capabilities. NiBNC was also examined for its reduction catalytic activity, and it was discovered to be more effective than pure BNC in reducing the amount of UV-absorption that a methyl orange solution had. Researchers looked into this catalyst’s reusability and found that it could be reused with good results even if it weren’t stored in an aqueous solution between uses.

Hexagonal phase CdS nanoparticles with outstanding photocatalytic activity were homogeneously coated onto the hydrated BNC nanofibers by a simple hydrothermal reaction at a temperature that was relatively low, and they were stabilized by means of the coordination effect. This reaction took place at a temperature that was in the range of around 100–200 °C. The CdS/BNC hybrid nanofibers demonstrated high efficiency photocatalysis, with 82% methyl orange breakdown after 90 min of irradiation and strong recyclability. According to the findings, it would appear that the CdS/BNC hybrid nanofibers are a good choice for application as highly stable photocatalysts that are responsive to visible light (Yang et al. 2011).

Synthesized through the decomposition of BC that was then infiltrated with an aqueous solution of zinc acetate while the process was carried out at a high temperature. In addition, using BC or Am-BC as a template, the photocatalytic

ZnO/BC and ZnO/Am-BC nanocomposite membranes can be synthesized using a simple polyol method. The resulting nanocomposites exhibit high photocatalytic activity in the degradation of methyl orange and good mechanical properties thanks to the inclusion of ZnO nanoparticles with a uniform distribution and an average diameter of 50 nm. ZnO/Am-BC nanocomposites with a high loading content of ZnO nanoparticles showed a higher decolorization efficiency of 91% at 120 min, making them a promising photocatalyst candidate.

A membrane technique referred to as superhydrophilic/underwater superoleophobic (SUS), which is used for the purpose of water filtration, has attracted a considerable amount of interest in recent years. Still, there is a major challenge to be met in the production of multifunctional membranes to meet the complex wastewater treatment requirements. The use of BNC-based multifunctional membranes for wastewater treatment is widespread. One type of nanocellulose employed in the creation of these multipurpose SUS membranes is bacterial nanocellulose (BNC). BC nanofibers and TiO₂ nanoparticles (NPs) were combined in order to produce membranes, and these membranes were further changed by the in situ growth of ZnO-Nps. Oil/water (o/w) separation has been demonstrated by the composite membranes at a flux rate of 8232.81212 L m²h⁻¹ and a high separation efficiency (>99.9%) under a low driving pressure (0.2–0.3 bar). Membranes, with a separation flux of 1498 74 L m²h⁻¹ and a high efficiency (99.25%), could also separate oil-in-water emulsion. To top it all off, the composite membrane displayed potent photocatalytic activity (>92%) when exposed to visible light. Inspiring the fabrication of next-generation multifunctional membranes for wastewater treatment, especially oily wastewater, dyes, and microbial contaminated water, is the high efficiency of the BNC based membranes (Wahid et al. 2022).

The use of novel photoactive membranes in treatment of wastewater and the elimination of microorganisms or organic pollutants from wastewater has drawn the attention of those working in the field of environmental protection. In addition to enhancing the adsorption and photocatalytic capabilities of such membranes, the concurrent application of BC with two distinct metal oxides or metal oxide-MWCNT nanocomposites could offer a promising alternative that could lengthen the lifetime of such membranes. Increasing the capacity for absorption is the strategy that would be used to bring about the desired outcome of achieving this goal. For example, BC-Fe₂O₃-ZrO₂ nanocomposites have been reported for the adsorptive removal of organic dyes and anti-pathogenic agents from contaminated water (Siddiqui and Chaudhry 2019) and BC-Fe₂O₃-SnO₂ nanocomposites have been reported for the same purpose (Siddiqui et al. 2019). The inorganic composite was manufactured using impregnation and solvothermal processes. Composite materials had 10 w/w% CTAB modified MWCNT added to them before use. The composites that resulted were given the names ZnO-MWCNT-IMP and ZnO-MWCNT-SOLVO to reflect the respective manufacturing processes. Methylene blue (MB) adsorption and photocatalytic degradation by bacterial nanocellulose-zinc oxide (ZnO)-multi walled carbon nanotube (MWCNT) hybrid membranes exposed to UV light. The photocatalytic performance of hybrid membranes is highly sensitive to both the preparation method and the amount of ZnO-MWCNT additives loaded into the

membranes. Under UV-irradiation and in filtration experiments, the SOLVO membranes exhibited significantly greater mechanical stability. In contrast, IMP membranes' lower tensile strength parameter led to failures in both photocatalysis and filtration experiments. Micrometer-sized ZnO particles in BC-ZnO-MWCNT-SOLVO membranes may therefore provide additional benefits and potential application alternatives, such as in the area of photocatalyst-based water treatment technology (El Mrabate et al. 2021).

In the past year, research into the removal of dyes and pollutants from water using heterogeneous catalysis led to the discovery of advanced oxidation processes, also known as AOPs. These processes are an additional method for cleaning water. Because of its distinct properties, the BNC is a promising substrate for developing a Fenton-type catalyst. To study the catalytic degradation of methylene blue (MB), a model dye pollutant, a Fenton-BNC hydrogel was freeze-dried to produce a Fenton-BNC aerogel. Its catalytic activity was higher than that of the bare Fenton catalyst made in the absence of BNC, suggesting that the heterogeneous Fenton catalyst supported by BNC is the superior catalyst. As an added bonus, Fenton-magnetic BNC's properties make it easy to recover with a magnet or magnetic field (Wibowo et al. 2018).

Owing to the fact that BC possesses both hydrophilic and oleophobic properties. Oil remains on the surface of the membrane during filtration of oily effluents or emulsions because only water droplets pass through the nanometric pores of the membrane at the pressure used in the process. Some research suggests that certain modifying agents can turn BC membranes hydrophobic, improving their oil/water selectivity for emulsions containing both oil and water (Sai et al. 2015). This is an effort to improve the filtration yield so that it conforms more closely to the specifications for the filtrate. Furthermore, BC is suitable for the removal of heavy metal ions from aqueous solution due to its nanoporous structure and its receptivity to chemical derivatization (Jin et al. 2017). To this end, it has been proposed to improve the adsorption capacity of cellulose-based materials by grafting them with functional groups like amino, carboxyl, and thiol (He et al. 2014). However, other reports have demonstrated that the ability of these modified materials to adsorb metal ions, as well as their mechanical properties, still require further improvement through new derivatization strategies. For this reason, research has been directed toward making these materials more flexible and mechanically strong than simple BC, the latter of which is crucial when these materials are used to create reusable membranes. The dense nanofibrillar structure of BC can hinder the performance of the separation process due to liquid infiltration, which can be mitigated by preparing composite materials with larger pore sizes. Because gravity alone is used for the separation process, no additional pressure is required, which means that less energy is used (Wang et al. 2021) (Fig. 4).

It is important to note that each of these alterations to the polymer can be carried out either independently or in conjunction with other systems. The few studies that have been done so far using BC as a filter membrane suggest that it has enormous unexplored potential, with particular reference to its low sensitivity to water, so that it does not decompose in contact with substances that are in liquid state, high degree

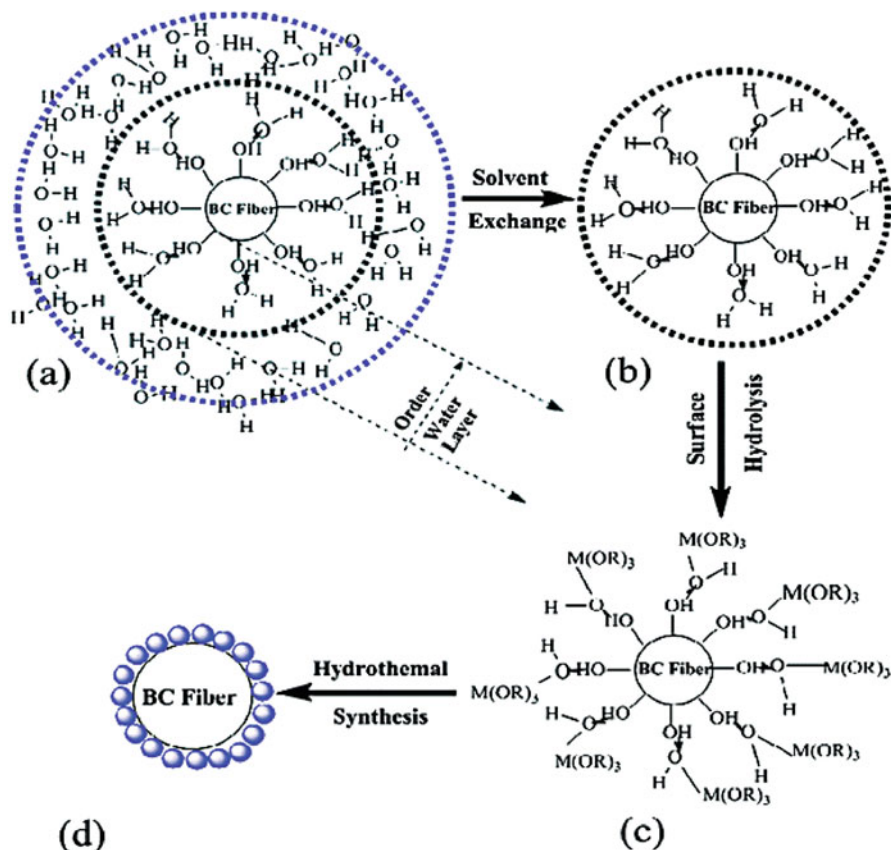


Fig. 4 Schematic illustration of the preparation of BC/TiO₂ hybrid nanofibers for the photo-degradation of model pollutant, methyl orange (MO) under UV irradiation (Sun et al. 2010b). Copyright@American Chemical Society, 2022)

of porosity, low density, and nanofiber structure that allow nanoscale filtration. In addition, its low sensitivity to water means that it does not decompose in contact with substances that are solid (Hungund and Gupta 2010).

A surprising level of efficacy in the orientation of TiO₂ nanoparticle arrays was demonstrated by bacterial nanocellulose (BNC) nanofibers. These nanofibers were biosynthesized by *Acetobacter xylinum* NUST5.2. We used a solvent exchange technique to gradually increase the ethanol concentration to up to 99%, which effectively removed the disordered water from the BNC solution. The BC fibers that had been treated were separated by centrifugation and then put into a 200 mL ethanolic solution of Ti(OBu)₄. The mixture was autoclaved in a stainless steel autoclave with a Teflon tube attached for 5 h at 150 °C (Fig. 5). The N-doped TiO₂/BC fiber composite underwent the same preparation method as the unmodified composite, except urea was added (Schaaff and Blom 2002).

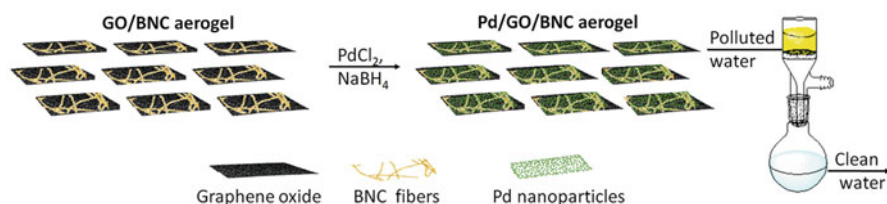


Fig. 5 Schematic illustration of the various steps involved in the fabrication of Pd/graphene oxide (GO)/bacterial nanocellulose (BNC) and its use towards remediation of water. (Reproduced from Xu et al. (2018)) Copyright@ Wiley-VCH, 2022)

By performing surface hydrolysis with molecular precision, it is possible to efficiently create large amounts of uniform BC nanofibers coated with TiO_2 nanoparticles. This process results in the development of uniform and well-defined hybrid nanofiber structures. Methyl orange (MO), employed as a model pollutant, was degraded using this TiO_2 /BNC hybrid nanocatalytic system as a photocatalyst, and it performed more effectively than the widely available P25 photocatalyst. The process for a series of photocatalytic degradation studies was as follows: materials were suspended (200 mg) in a MO solution (20 ppm) and put in a 200 mL quartz reactor with a 300 W UV light. In addition to the commercial P25, the findings of comparing BNC/ TiO_2 hybrid nanofibers with BC/ TiO_2 hybrid nanofibers doped with nitrogen have been compared (Sun et al. 2010b).

In situ reduction of Ag^+ by $\text{Na}_3\text{citrate}$ on the BC matrix was used by Marques et al. to create an AgNP/BNC nanocomposite. Thiosalicylic acid and 2,2'-dithiodipyridine can both be detected using the resulting AgNP/BNC substrate at concentrations as low as 104 M. Additionally, this substrate has been used to acquire the SERS spectra of three amino acids: L-phenylalanine, L-glutamate, and L-histidine. The hydroxyl groups of BC reduced HAuCl_4 in situ at 373 K to create AuNPs in the interstitial space of the layer of BC hydrogen, resulting in an Au/BNC nanocatalyst (Marques et al. 2008). For the SERS detection of trace organic compounds such as phenylacetic acid and 4-fluorobenzenethiol (4-FBT), Park et al. created AuNP/BC substrates (PAA). SERS hot spots were produced as the hydrogel dried and the space between its surrounding layers and the attached AuNPs significantly shrunk. This method allowed for the detection of PAA at low concentrations (107 M). It is noteworthy to note that the minimum concentration detected for 4-FBT, which has a high affinity for AuNPs, was much higher (105 M). This strategy profited from BC's three-dimensional structure. Drying-mediated deformation of the three-dimensional AuNP/BC nanocomposites produced hot spots in the vertical direction that resulted in significantly stronger Raman signals than standard two-dimensional SERS substrates (Park et al. 2013).

Ferreira-Neto and colleagues developed self-supported hybrid aerogel membranes of bacterial nanocellulose/MoS₂ nanocomposite systems, which combine the mechanical stability and remarkable textural properties of bacterial nanocellulose (BNC)-based organic macro/mesoporous scaffolds with the superior adsorption-cum-photocatalytic capabilities and high pollutant removal performance of MoS₂

nanostructures. Coating quantum-confined photocatalytic MoS₂ nanostructures (2–4 nm crystallite size) on BC nanofibrils through controlled hydrothermal growth and exact adjustment of the synthetic parameters allowed us to construct BC/MoS₂-based porous, self-supported, and stable hybrid aerogels with a distinctive shape. These BC/MoS₂ samples feature significant surface area (97–137 m²g⁻¹), small pore volume (0.28–0.36 cm³ g⁻¹), and short interlayer distances (0.62–1.05 nm). Researchers found that by modifying BC with nanostructured MoS₂, the hybrid aerogels' efficiency at removing pollutants via adsorptive and photocatalytic mechanisms was greatly improved. This was demonstrated in a comprehensive study using a membrane photoreactor specifically designed to house the developed photo-active/adsorptive BC/MoS₂ hybrid membranes. Both organic dye (methylene blue (MB)) molecules (96% removal within 120 min, $K_{\text{obs}} = 0.0267 \text{ min}^{-1}$) and heavy metal ions (88% removal within 120 min, $K_{\text{obs}} = 0.0012 \text{ min}^{-1}$) were efficiently removed via photoassisted in-flow removal using the prepared BC/MoS₂ aerogel membranes, demonstrating excellent recyclability and photo-stability. According to extensive environmental studies, the interlayer expanded MoS₂ nanostructures were much more successful in the removal of smaller species (CrO₄²⁻) as compared to larger (MB) dye molecules. This was the case in both cases when comparing the two different sizes of dye molecules. The created hybrid aerogel membranes display promising behavior for use in in-flow water purification, representing a significant advance over the use of self-supported aerogel membranes for photocatalytic applications in liquid media (Ferreira-Neto et al. 2020).

Synthetic Applications of BNCs Grafted with Inorganic Catalysts

Because of its desirable features, such as great mechanical strength and toughness, rich chemical functionality, and high specific surface area in aerogel state, bacterial nanocellulose (BNC) serves as an appropriate material for realizing functional composites by adding functional nanomaterials inside BNC matrix. These properties include: outstanding mechanical strength and toughness, rich chemical functionality, and high specific surface area in aerogel state. The aerogel state exhibits several ideal qualities, including a large specific surface area, great mechanical strength and toughness, and a rich chemical functionality. Graphene oxide (GO) sheets and other 2D materials can be efficiently included into the growing matrix of BNCs. Durable membranes, like those made possible by the efficient intercalation of GO flakes within the layered BNC matrix, are crucial for efficient, large-scale water treatment. Accordingly, Jiang et al. have demonstrated a simple and repeatable procedure for constructing Pd/GO/BNC membrane for very effective dye degradation. A Pd/GO/BNC membrane is made by first growing a BNC matrix with GO flakes inserted into the matrix, and then growing PdNPs directly on top of the GO flakes. The layered structure of Pd/GO/BNC produces nanocapillaries throughout the membrane, bringing organic dye pollutants closer to the PdNPs anchored on the GO flakes. These innovative membranes show promise for use in wastewater treatment and separation due to their efficiency in degrading organic dyes.

Realization of this Pd/GO/BNC membrane is made possible by in situ incorporation of GO flakes into the BNC matrix during its growth, followed by in situ formation of palladium nanoparticles (Fig. 5). This membrane exhibits extremely efficient methylene orange (MO) degradation during filtration (up to 99.3%) over a broad range of MO concentrations, pH, and multiple cycles of reuse. Pd/GO/BNC membrane has proven successful in treating numerous pollutants at once (a cocktail of 4-nitrophenol, methylene blue, and rhodamine 6G). The new and strong membrane developed in the paper shows promising results in wastewater treatment. The efficiency of degradation remained stable at 99.3% throughout a broad range of pH values encountered in both natural and manufactured aquatic systems. The Pd/GO/BNC membrane suffered just a minor performance loss (to 99.2%) after being filtered and regenerated six times (Xu et al. 2018).

Porous crystalline materials called metal organic frameworks (MOFs) can be designed to act as selective adsorbents. Because of their great porosity, they can absorb a lot of liquid. However, the brittleness of these crystalline materials presents problems for many commercial applications. Liquid phase separation techniques can benefit from the usage of MOFs that have been immobilized on solid supports and scaled up for commercial application. Nanocellulose is a promising candidate for use in this context due to its excellent flexibility and biocompatibility. The Fe(III) 1,3,5-benzenetricarboxylate-containing MIL-100(Fe) from the Material Institute de Lavoisier was immobilized onto bacterial nanocellulose (BNC) nanofibers to create a new BNF-MOF nanocomposite. The nanofibrils of bacterial cellulose were extracted from a pellicle of uniformly developed bacteria using 30 vol% reagent grade acid (Merck KGaA, Stockholm, Sweden) in 1 L of aqueous sulfuric acid solution with stirring at 60 0.5 °C. (300 rpm). When using the new nanocomposite material for efficient arsenic and Rhodamine B separation from aqueous solution, adsorption capacities of 4.81 and 2.77 mg g⁻¹, respectively, were achieved. In order to conduct adsorption studies, 0.18 grammes of MIL-100(Fe)@BC was dissolved in aqueous solutions containing various pollutants at concentrations ranging from 20 mg L⁻¹ of As(III) (50 mL) to 10 mg L⁻¹ of Rhodamine B (40 mL) for a set amount of time. Following the extraction of the MIL-100(Fe)@BC nanocomposite from the aqueous solution, a UV-vis examination of the dye concentration was carried out in accordance with a calibration curve. Nonlinear pseudo-second-order fitting provided a good model for the adsorption process. Meanwhile, inductively coupled plasma atomic emission spectroscopy was used to quantify the amount of As(III) in the supernatant (ICP-OES) (Ashour et al. 2020).

Conclusions and Future Prospects

Concerns for the environment, sustainability, reusability, and renewability of conventionally utilized materials have led to a greater focus on the development of novel materials for cutting-edge applications in the research of nanomaterials. Based on these features, BNC is a suitable construction component for the manufacture of emerging materials. Researchers are increasingly interested in BNC-supported

catalysts due to its many desirable properties, including low toxicity, excellent catalytic holding power, and, most crucially, ease of manufacture in comparison to plant-based nanocellulose. The mechanical, chemical, porous, water-absorbing, moldable, biodegradable, excellent, and biological affinity properties of BNC are unparalleled. These characteristics, in particular when applied to inorganic systems, give rise to a broad spectrum of possibilities for the functional alteration of the system. Production, physical-chemical characteristics, functionalization, especially with inorganic nanosystems, and new developments in BNC-based catalysis are discussed in this chapter. Topics covered include reduction, oxidation, coupling, electrocatalysis, and photochemical reactions. Recent research is capitalizing on the superior performance of BNC-based nanocatalysts in a variety of applications, including reduction reactions, oxidation reactions, photocatalysis, electrocatalysis, wastewater remediation treatments, and environmental bioremediation reactions. Inorganic nanoparticle-modified BNCs are highly effective catalysts for a wide variety of processes. The Heck, Ullmann, Suzuki, and A3 couplings are examples of important coupling reactions; (b) reduction reactions using Au, Ag, Pd, Au-Pd, Pt, Cu, Ni, and CuO NPs supported onto BNCs or composite catalysts have been successfully studied over the past few decades; and (c) oxidation reactions using Pd-based catalysts are examples of important oxidation reactions.

In light of the many advantages associated with bacterial nanocellulose, it is essential to acquire more information regarding the ideal loading levels of the numerous NPs that comprise this matrix. Nanocellulose's support from the matrix can be short-circuited over the long haul if it contains inorganic ingredients. If nanocomposites are to be used indefinitely, researchers will need to find ways to either lengthen their service life or replenish them. Nanocomposites based on nanocellulose can be an attractive alternative when seeking for a low-cost, eco-friendly material to address growing environmental concerns. Furthermore, it is difficult to avoid its disintegration because it is utilized as reinforcing filler and has poor compatibility with other hydrophobic polymers. It has a high production cost and a lower yield compared to the extraction method used to generate nanocellulose from other sources, and its high hydrophilicity reduces cellular adhesion and proliferation compared to other protein-based materials.

In the not-too-distant future, it is possible that surface functionalization utilizing cutting-edge technologies may be able to improve and enhance the nature of BNC while simultaneously fixing its limitations. This would be a significant step forward. More and more studies are using BNC-based products as stabilisers, polymer additives, functional catalysts, elements of energy storage materials, medication administration, self-healing, and stimulus sensitive materials. In the field of medicine, the application of BNC and materials based on BNC has led to significant shifts as a result of the modifications. In recent years, scientists have used genetic engineering to develop a wide variety of bacteria with novel properties. The special capacities of these bacteria will soon allow for the production of BNC with better properties, which should match the needs of producing cutting-edge bio-based functional materials for novel applications.

In conclusion, water-based nanocellulose suspensions offer a stable system in which biphasic catalysis can be investigated. The metal NPs and salts in these suspensions are exposed to conditions similar to those seen in ionic liquids. Based on it, another significant approach to follow would be to investigate the ligand exchange processes employing organometallic precursors in the CNC-water solution. The resulting system could be tested for catalysis in several processes of interest, such as C-H activations, C-C coupling reactions, redox reactions, etc. To sum up, we find that the usage of nanocelluloses in catalytic applications has led to the development of intriguing new catalytic systems over the past few years. New and valuable applications based on these insights are expected to emerge eventually.

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Jorge Padrão, Liliana Melro, Marta Fernandes, Rui D. V. Fernandes, Ana Isabel Ribeiro, Xinyu Song, Liangmin Yu, and Andrea Zille

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Abstract

Nanocellulose already proved to be a highly relevant material for biomedical applications, ensued by its outstanding mechanical properties and, more importantly, its biocompatibility. Nevertheless, despite their previous intensive research, a notable number of emerging applications are still being developed. Interestingly, this drive is not solely based on the nanocellulose features, but also heavily dependent on sustainability. The three core nanocelluloses encompass cellulose nanocrystals (CNCs), cellulose nanofibrils (CNFs), and bacterial nanocellulose (BNC). All these different types of nanocellulose display highly interesting biomedical properties per se, after modification and when used in composite formulations. Novel applications that use nanocellulose include

J. Padrão (✉) · L. Melro · M. Fernandes · R. D. V. Fernandes · A. I. Ribeiro · A. Zille (✉)
Centre for Textile Science and Technology (2C2T), University of Minho, Guimarães, Portugal
e-mail: padraoj@2c2t.uminho.pt; liliana.melro@2c2t.uminho.pt; marta.fernandes@det.uminho.pt; afr@2c2t.uminho.pt; azille@2c2t.uminho.pt

X. Song · L. Yu
Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Qingdao, China
e-mail: xinyu.song@ouc.edu.cn; yuyan@ouc.edu.cn

well-known areas, namely, wound dressings, implants, indwelling medical devices, scaffolds, and novel printed scaffolds. Their cytotoxicity and biocompatibility using recent methodologies are thoroughly analyzed to reinforce their near future applicability. By analyzing the pristine core nanocellulose, none display cytotoxicity. However, CNF has the highest potential to fail long-term biocompatibility since it tends to trigger inflammation. On the other hand, never-dried BNC displays a remarkable biocompatibility. Despite this, all nanocelluloses clearly represent a flag bearer of future superior biomaterials, being elite materials in the urgent replacement of our petrochemical dependence.

Keywords

Nanocellulose · Biomedical · Bacterial nanocellulose · Cellulose nanofibrils · Cellulose nanocrystals

Introduction

Fortunately, the amount of research focused on the use of nature-based substrates in wound dressings, indwelling devices, and other medical textiles has been subject to an impressive exponential growth. Nevertheless, in recent years due to the COVID-19 pandemic, there is an unprecedented boom in demand for petrochemical-based medical textiles to produce a staggering amount of personal protective equipment, in particular masks and respirators. With the deceleration of the pandemic allied to the ever more evident climate change-driven phenomena, the sustainability concerns are becoming an essential feature of every material or textile (Padrão et al. 2022). Furthermore, in biomedical applications the use of biopolymers is not only due to sustainability concerns, but majorly due to their biocompatibility. Biocompatibility may be briefly summarized as the impact of a medical application on the host. If it triggers an adequate or benefic response by the host, it is considered biocompatible. However, if it cascades into an ominous reaction, such as necrotic foreign body granulomas, the application or material is classified as nonbiocompatible (Barrère et al. 2008). Therefore, relevant biocompatibility properties include the assessment of the cytotoxicity of the novel applications developed *in vitro*, prior to experiment *in vivo*. Cytotoxicity may simplistically be regarded as how the materials will impact the living cells in terms of viability, proliferation, metabolism, adhesion, morphology, expression of key metabolites or signals, among others. If the material has a clear negative impact on one or several of these parameters, it will be classified as cytotoxic (Ramakrishna et al. 2015). The established threshold for acceptable loss of viability is 30% (Montanaro et al. 2000). If a material is considered cytotoxic, then it will most likely fail to exert an adequate or beneficial host response, thus failing to be biocompatible. If the material is considered noncytotoxic, it will possess a higher probability to trigger a suitable response by the host. However, a noncytotoxic material may still fail to be biocompatible (Fig. 1). In addition, hemocompatibility is a key parameter for medical applications. Hemocompatibility of a material is

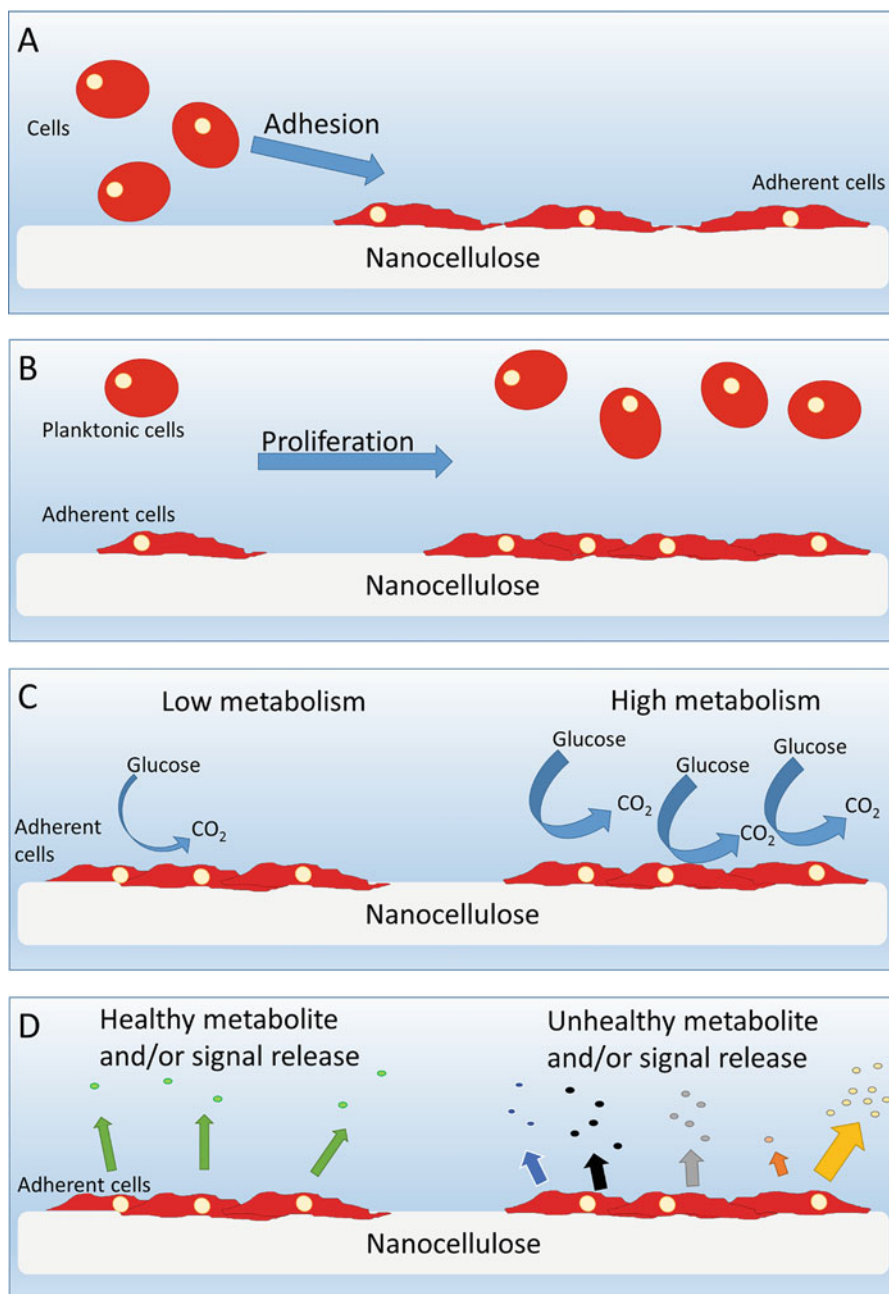


Fig. 1 Nanocellulose-based materials. Examples of cytotoxicity parameters: (a) cell adhesion, (b) cell proliferation, (c) cell metabolism, (d) metabolite and/or cell signaling, and (e) cell morphology. Examples of biocompatibility parameters: (f) tissue viability and (g) tissue signaling

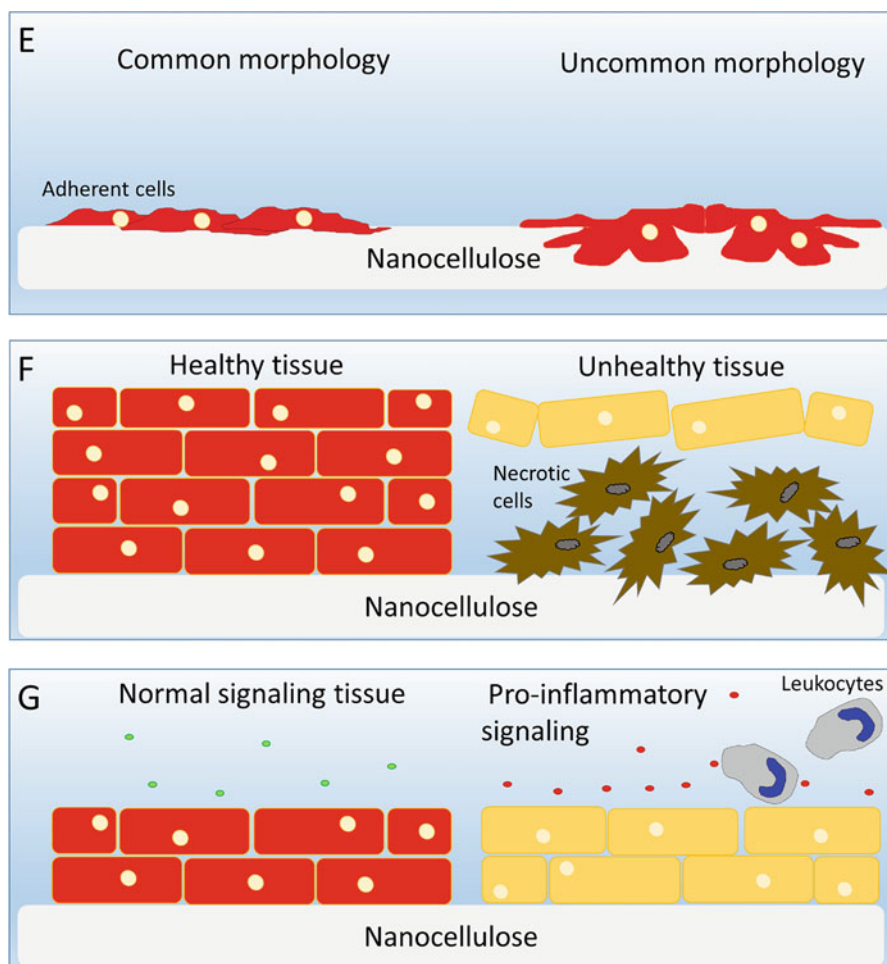


Fig. 1 (continued)

defined by its propensity to lyse erythrocytes, as well as by its level of adsorption of coagulating agents and other signaling molecules that interact with the complement activation pathway. Cellulose is understandably one of the most common biopolymer substrates for biomedical applications and medical textiles due to its ubiquitous availability and remarkable properties. Cellulose has been used since the beginning of human history; however, cellulose and cellulose-based materials underwent an important revolution with the advent of nanotechnology. Nanotechnology was defined by the scientist Norio Taniguchi in 1974, which ensued the thriving development of nanoscale materials, which depict 1–100 nm in at least one of their

dimensions (Bayda et al. 2020). Nanocellulose encompasses five main types: cellulose nanocrystals (CNCs), cellulose nanofibrils (CNFs), bacterial nanocellulose (BNC), electrospun nanocellulose (ESNC), and wet-spun nanocellulose (WSNC). In brief, CNCs are generated through the chemical hydrolysis of the amorphous regions of cellulose. The crystalline cellulose, which is able to withstand the aggressive chemical hydrolysis, is collected and purified. Commonly, the smallest size of CNCs is approximately 3 nm wide and 70 nm long, and the largest reported size is nearly 25 nm wide and 150 nm long. Due to their needle shape, allied to this size ratio, CNCs are also named whiskers. CNFs are obtained from mechanical degradation of the amorphous regions, and this process may be aided by the presence of enzymes or chemicals. Unlike CNC, CNF in scanning electron microscopy (SEM) images depicts a “spaghetti”-like appearance due to their fairly higher length ranging from approximately 500 nm to over than 1 μ m. CNF width is encompassed between 20 and 60 nm. Both CNC and CNF may be obtained from a plethora of origins, encompassing nearly all sources of cellulose (including BNC). The BNC is mainly synthesized from bacteria. BNC nanofibers are excreted by bacteria. During incubation, the buildup of generated fibrils originates in a three-dimensional matrix. The BNC fibril size is influenced by several parameters, namely, bacteria strain, dissolved oxygen concentration, type of culture (static or dynamic), incubation time, reactor layout, culture media, among other parameters. BNC crystallinity is fairly superior to plant cellulose. Furthermore, it is solely composed of cellulose, thus to obtain a 100% nanocellulose material, the only purification procedure required is to remove bacteria, bacteria debris, and culture media constituents from the collected BNC (Padrão and Zille 2021). The most common purification consists of a maceration procedure. This purification procedure reduces the liposaccharide concentration values considerably below the threshold defined by the Food and Drug Administration (Gonçalves et al. 2015). Therefore, the size of BNC fibers and their concentration (which influences pore size) is highly variable: width between 10 to 100 nm and 50 nm up to 100 μ m in length. CNC, CNF, and BNC may be regarded as core nanocellulose materials since the ESNC and WSNC are obtained from them. In brief, electrospinning consists of the production of a fiber (usually at nanoscale) that is extruded through the generation of a potent electrical field between the spinneret and a grounded collector. During their flight, the fibers crosslink. For ESNC, one or a combination of the previously mentioned nanocelluloses is present in the dope solutions. Similarly, WSNC uses one or a combination of the above-described nanocelluloses as one of the components added to a mixture that is extruded (usually using mild conditions) into a coagulation bath where the components crosslink. The nanosize of nanocellulose greatly enhances the availability of the hydroxyl groups that populate their surface. Fibrinogen, a glycoprotein present in blood, may be cleaved in an array of different peptides that regulate cell adhesion, cell migration, and may work as chemotactic for key angiogenesis cells such as fibroblasts, keratinocytes, macrophages, myocytes, and endothelial cells. Fibrinogen bonds tightly to -OH groups (Thevenot et al. 2008). Thus, the outstanding -OH availability in nanocellulose may result in a favorable promotion of angiogenesis. In

addition, the impressive nanocellulose crystallinity may be explored in the development of chemically and mechanically sturdier materials for non-host-degradable applications. These features will be critically analyzed in this chapter. Furthermore, the emerging applications of nanocellulose in the medical field will be discussed according to its enhanced biocompatibility.

Cellulose Nanocrystals and Cellulose Nanofibrils

Nanosized cellulose has been widely studied due to the unique properties, namely, its surface chemistry, mechanical properties, biocompatibility, biodegradability, and wide availability. CNC and CNF present similar chemical composition but different morphologies owing to the differences in their manufacturing procedures. CNCs are short, rod-like cellulose nanocrystals, whereas CNFs are long entangled cellulose crystalline fibrils. CNFs exhibit remarkable gelling properties in water. Furthermore, CNF displays superior reinforcing properties in polymer nanocomposites in comparison to CNC (Ghafari et al. 2019; Svagan et al. 2014). CNCs were proved to be noncytotoxic to a plethora of cell lines, despite the high concentrations tested ($50 \mu\text{g mL}^{-1}$) (Dong et al. 2012). Cytotoxicity of CNF was investigated using human monocytic (THP-1) cells, without any signs of cytotoxicity even using high doses ($100 \mu\text{g mL}^{-1}$). However, its exposure did trigger a higher release of growth factors and relevant pro-inflammatory cytokines, which may induce a problematic inflammatory event (Bhattacharya et al. 2017). Ho et al. studied the long-term in vivo biocompatibility of CNC and CNF through their subcutaneous implantation in Sprague–Dawley rats. No foreign body reaction was observed up to 2 weeks after implantation. No indication of implant rejection was observed in rats that were observed for CNC or CNF materials. Histologically, CNC exhibited an active cell migration; however, some acute inflammatory effects were also noticed. CNF subcutaneously implanted denoted the presence of fibrotic capsule, indicating chronic inflammation signs after 3 months. These findings were corroborated by the thickness of fibrous tissue formed around the injected nanocellulose materials. The thickness of fibrous tissue in CNC increased within the first 2 weeks and subsequently declined. Whereas CNF showed an increasing thickness of the fibrous tissue unfolded during the first month and remained stable afterward. The increase of fibrous tissue in CNF could lead to granulation, which may elicit a long-term foreign body response, due to CNF slow degradation (Ho et al. 2020). Therefore, in comparison, CNC displays a higher potential for biomedical applications than CNF. Nevertheless, and as an example, the same study describes superior biocompatibility once CNF is oxidized using 2,2,6,6-tetramethylpiperidinyloxy (TEMPO) (Ho et al. 2020). Thus, both CNC and CNF remain landmark materials for the development of emerging biomedical applications, per se, modified or as part of a composite formulation. These applications include tissue engineering and wound dressing (Table 1).

Table 1 CNC and CNF emerging biomedical applications properties

CNC/CNF source	Functionalized nanocellulose/ "composite"	Adequacy	Assays	Cell/animal	Application	References
CNF, bleached softwood kraft pulp (a mixture of pine (25%) and spruce (75%))	TEMPO-oxidation and carboxymethylation of CNF	(a) In vitro – cytotoxicity (b) In vivo – biocompatibility	(a) Cell viability, proliferation, and morphology on scaffolds (live/dead staining, DNA assay); inflammatory cytokine measurement (Bio-Plex Human 27-plex kit) (b) Cytokine measurements (BCA Protein Assay Kit)	(a) Immortal human monocyte/macrophage (U937 cells) (b) Rats	Drug delivery and tissue engineering	Rashad et al. (2019)
CNC, commercial	Methacrylated gellan-gum and CNC	In vitro	Cell viability and morphology (live/dead assay)	Encapsulated annulus fibrosus cells	Annulus fibrosus tissue regeneration	Pereira et al. (2018)
CNC, commercial	APTES silylation of CNC and carboxylated nanodiamonds	In vitro	Cell morphology (live/dead assay), adhesion (live/dead assay) and differentiation (fluorescence microscopy)	Human fetal osteoblasts (cell line hFOB 1.19)	Bone tissue regeneration	Vega-Figueroa et al. (2017)
CNF, birch fibers	Quince seed mucilage and TEMPO-oxidation of CNF	In vitro	Cell proliferation (WST-1 assay)	Human liver cancer (HepG2 cells)	Scaffolds for soft tissue engineering	Baniasadi et al. (2021)

(continued)

Table 1 (continued)

CNC/CNF source	Functionalized nanocellulose/“composite”	Adequacy	Assays	Cell/animal	Application	References
CNC, <i>Eucalyptus</i> pulp	TEMPO-oxidation of CNF and mixture with polyethylene glycol diacrylate	In vitro	Cell viability (MTT assay)	Normal mouse embryonic fibroblast cells (NIH3T3 cells)	Aerogel scaffold for tissue engineering	Tang et al. (2019)
CNC and CNF, commercial	CNC, CNF, and TEMPO-oxidized CNF	(a) In vitro (b) In vivo	(a) Cell viability (MTT assay) (b) Foreign body reaction (microscopy)	(a) Fibroblast cells (L929) (b) Rats	Drug delivery, bone regeneration	Tang et al. (2019)
CNC, Whatman® cotton filter	CNC-eg-PEO	In vitro	CCK-8 assay	ATCC cell lines of human foreskin fibroblasts (HFF) and human tongue squamous carcinoma (CAL-27)	n.d.	Zhao et al. (2021)
CNC, Whatman® cotton filter	CNC-β-CD	In vitro	SDS-PAGE, Immunoblotting, ELISA	Cell lines of mouse macrophage-like J774A.1 and human leukemia monocyte THP-1	Drug delivery	Sunasee et al. (2019)
Purified cotton, commercial	Aligned and random ECCNNs loaded with bone morphogenic protein-2 (rhBMP-2)	(a) In vitro (b) In vivo	a) MTS assay (osteogenic differentiation) (b) bone regeneration	Bone marrow-derived Stem cells (BMSCs)	Tissue engineering	Zhang et al. (2019)
CNF, commercial	CNF/LCC-48; CNF/LCC-72 (LCC = lignin carbohydrate complex)	In vitro	Cell growth with scaffold	Human coronary artery endothelial cells (HCAECs)	Tissue engineering scaffolds	Zhang et al. (2019)

CNF, birch kraft pulp	CNF modified with dehydroabietic acid derivatives	In vitro	(a) Cell viability (live/dead assay) and (b) toxicity toward blood cells (absorbance measurements) Cell viability (MTT assay)	a) Human skin fibroblasts and (b) blood from healthy human donors Normal mouse embryonic fibroblast cells (NIH3T3 cells)	Wound healing	Hassan et al. (2020)
CNC, <i>Hibiscus cannabinus</i> fibers	Chitosan/poly (vinyl pyrrolidone)/nanocellulose	In vitro	Cell viability (MTT assay)	Normal mouse embryonic fibroblast cells (NIH3T3 cells)	Wound healing	Poonguzhali et al. (2017)
CNF, commercial	CNF and lysozyme nanofibers	In vitro	Antioxidant activity (DPPH radical-scavenging method); cell viability (PrestoBlue assay); cell adhesion (SEM and live/dead assay); migration capability of fibroblasts (microscopy)	Fibroblast cells (L929)	Wound healing	Silva et al. (2020)
Microcrystalline cellulose	Gelatin (GA); Hyaluronic acid (HA); HA-GA; GA-HA-CNC GA-CNC; HA-CNC	In vitro	Cell proliferation (CCK-8 assay) Cell viability (live/dead assay)	Cell line of mouse fibroblasts (NIH3T3)	Wound healing	Yin et al. (2019)

n.d., not defined

Tissue Engineering

Tissue engineering was defined over 30 years ago and has been evolving to describe multidisciplinary approaches in the development of materials that are able to recuperate, maintain, or enhance the tissue or organs' healthy operation (Chapekar 2000). A research work developed by Rashad et al. investigated the inflammatory response of porous hydrogel scaffolds made of CNF oxidized by TEMPO or by CNC carboxymethylation. The biocompatibility and inflammatory response of the hydrogels were assessed *in vitro* using immortal human monocyte and macrophage U937 cells, and *in vivo* using rats. Host responses to the hydrogel scaffolds were evaluated within a 1-month period. Furthermore, the foreign body reaction was evaluated through histological analysis for half a year. Tissue plate culture (TCP) and gelatin scaffolds were employed as controls. After 3 days, no significant differences were observed in the production of chemokines growth factors and pro-inflammatory cytokines between the CNF scaffolds and TCP. These important signals were quantified through polymerase chain reaction (PCR) and Bioplex bead arrays. However, on the fourth day *in vivo*, a significantly higher gene expression of the anti-inflammatory interleukin (IL)-1Ra in the modified CNF scaffolds was clearly seen when compared to the gelatin scaffold. After 1 month, CNF scaffolds clearly stimulated the production of IL-10, an anti-inflammatory cytokine. Unlike gelatin, both CNF scaffolds had not degraded after 6 months. The slow degradation (or even absent degradation) of the modified CNF scaffolds triggered a foreign body reaction. CNF scaffold surface chemistry promptly triggered cytokine production and did not modify the inflammatory profile *in vitro* or *in vivo*. Clearly, both modified CNF scaffolds did not display any cytotoxicity *in vitro* or *in vivo*. Moreover, they induced repair processes, an important feature for patients that display impaired wound healing. Nevertheless, to promote a transition from reparative to regenerative properties, further functionalizations of CNF materials are required. The authors suggested an accelerated degradation of the scaffolds to prevent the foreign body reactions (Rashad et al. 2019). Pereira et al. proposed a methacrylated gellan gum-based scaffold reinforced with CNC as a self-gelling fibrous that may be used as an injectable. The hydrogels were characterized regarding the swelling capacity, degradation rate *in vitro*, and mechanical properties. Rheological evaluation of the nanocomposites demonstrated that the gellan–gum reinforcement with CNC promoted matrix entanglement with higher scaffold stiffness due to ionic crosslinking. Mechanical tests demonstrated a compressive modulus value close to those of the human annulus fibrosus tissue. Furthermore, cell culture studies with encapsulated bovine annulus fibrosus cells indicated that the developed nanocomposite promoted cell viability and maintained adequate cell morphology for up to 14 days *in vitro* (Pereira et al. 2018). Vega-Figueroa et al. assessed a CNC–nanodiamond composite as eclectic application for the regeneration of bone tissue. The authors assessed surface functionalization, biocompatibility, and impact on human fetal osteoblastic cell (hFOB) proliferation and differentiation. CNC was functionalized via a 3-aminopropyltriethoxysilane silylation. Afterward, a strong acid oxidation was used to generate carboxyl groups on the surface of the

nanodiamonds. Then, an amide linkage promoted to covalently bound the two. The nanocomposite was tested for its suitability as a material interface for cell growth, in particular its chemical compatibility. hFOB cell morphology, adhesion, differentiation, and expression of osteocalcin and sialoprotein were evaluated. Each component used in the fabrication of the nanocomposite was tested. No striking differences were observed between the material and their combinations. The immunocytochemical analysis showed no detrimental morphological differences of hFOB cells (which are commonly elongated and polygonal), in the expression of osteocalcin and sialoprotein or cell viability. The results reinforced the concept that micro-/nanotopography may considerably improve the osteoblast adhesion, avoid unfavorable differentiation, and promote bone formation. This may be due to the micro-/nanotopography structure that has the potential mimic the topography of the extracellular matrix (Vega-Figueroa et al. 2017). Baniasadi et al. developed new viscoelastic bioinks composed of quince seed mucilage and CNF, which were extruded by direct ink writing into three-dimensional (3D) lattice scaffolds. The scaffolds denoted suitable porosity, water uptake capacity, and mechanical strength for tissue engineering, in particular scaffolds for soft tissues and wound dressings. All samples exhibited a rapid water uptake during 2 h, and dramatically diminished up to 24 h. They presented high swelling ratio, similar to the values reported for hydrogels used in biomedical applications. The scaffolds' high porous structure may have favored water diffusion within the homogeneously porous internal structure. By increasing CNF content, the scaffolds displayed a denser and tightly organized network, with smaller pore size and higher surface area. Moreover, CNF slowed the degradation rate, due to its high crystallinity. Several tissues require a longer period of time for re-epithelialization, namely, the skin. Thus, hydrogels that denote lower degradation rate and higher physical stability could be an important asset for this organ. The incorporation of CNF increased the compressive modulus and ultimate strength by approximately 50% and 70%, respectively, indicating a stiffer hydrogel and a reinforcing function of CNF. The obtained hydrogels displayed mechanical properties comparable to the skin and soft tissues. Human liver cancer (HepG2) cells were used to assess the biocompatibility of the printed samples. The results showed no cytotoxicity and suitability to promote attachment and proliferation of the cells. CNF loading and cell viability increased proportionally, which can be explained by the higher porosity, swelling ratio, and physical integrity of the structures with higher CNF concentration. Enhancing the surface area available for cell proliferation by the presence of CNF was found to be essential for filament self-supporting and shape retention after deposition (Baniasadi et al. 2021). Most of the printed nanocellulose scaffolds still exhibit limited resolution and mechanical properties due to the lack of CNF curing properties or thermoplasticity to form shape. Therefore, the development of 3D nanocellulose natural polymer with desired precision and mechanical strength for tissue engineering remains a challenge. Blending CNFs with photocurable resins is a strategy to solve their compatibility to obtain a stable and uniform suspension with proper rheology and photocuring properties. Tang et al. mixed cellulose nanofibers and polyethylene glycol diacrylate. The CNF with a waterborne photopolymerizable acrylic resin was used to fabricate 3D cytocompatibility

hydrogel scaffold by stereolithography process. CNFs were prepared by TEMPO-oxidation combined with mechanical treatment to disperse uniformly the CNF in water forming a transparent suspension due to the electrostatic repulsion of carboxyl groups. The excellent mechanical reinforcement of CNF with other matrixes results from their high specific surface area and numerous hydrogen and carboxyl groups. The produced hydrogels were all transparent and homogeneous. The hydrogel scaffolds possess high and tunable compressive modulus and high porosity of approximately 90%. The CNF content exhibited a significant role in structural shape integrity, porous structure, and mechanical strength. The biocompatibility tests using mouse fibroblasts (NIH3T3) showed a tight adherence to the composite, adequate differentiation, and viability. The scaffolds containing CNF showed an obvious increment of NIH3T3 cells, which was not observed on the scaffolds without CNF. This difference may be related to the porous networks of the CNF-containing scaffold, which provide more anchor points for the cells. Whereas pure polyethylene glycol diacrylate scaffolds due to their flat surface did not favor as much the cell adhesion. These results have revealed a superior method to prepare tissue engineering scaffolds that possesses suitable mechanical strength and biocompatibility for 3D cell cultivation (Tang et al. 2019). Zhao et al. prepared CNC from Whatman® filters, which were activated by thiolation, and then end-grafted with polyethylene oxide (PEO), to form a composite. Pristine CNC and the developed composites were redispersed in aqueous solution for 20 days. The thiolated CNC-containing polyethylene oxide composite exhibited a high dispersion stability without forming any sedimentation. The cytotoxicity of the modified CNC was estimated by the viability of human foreskin fibroblasts (HFF) and human tongue squamous carcinoma (CAL-27) cells. No statistical difference in viability was detected between the control group and modified CNC composites. These results reflected the low cytotoxicity of these CNC composites, indicating their potential as carriers of bioactive agents (Zhao et al. 2021). Sunasee et al. also produced CNC from Whatman® filters, which were functionalized with β -cyclodextrins (B-CD) to study their immunological and antioxidant response in (THP-1) and mouse macrophage-like cells. Furthermore, an immunological study was performed by tracking the secretion of inflammatory cytokine interleukin-1 β (IL-1 β) in myeloid cell lines. It was observed that the obtained CNC composites did not induce a strong immunological response in either THP-1 or J774A.1 cells. However, the CNC containing β -cyclodextrins showed a slight propensity to enhance IL-1 β secretion in human cells at higher concentrations. In addition, no major oxidative stress was observed (Sunasee et al. 2019). Zhang et al. used electrospinning to generate aligned and unaligned/random cellulose and CNC-based ESNs, to be used as scaffolds. These ESNs were coated with recombinant human bone morphogenetic protein-2 (rhBMP-2). Bone marrow-derived stem cells (BMSCs) were isolated from Wistar rats and cultured, firstly by themselves and then with the CNC-based ESN scaffold. Cell growth, viability, and distribution among the scaffolds were assessed, which exhibited no significant difference in proliferation between the control, aligned or random ESN, and aligned or random ESN functionalized with rhBMP-2. However, BMSC depicted a distinct morphology according to the

scaffolds. BMSCs were spindle in aligned scaffolds and polygonal when incubated in random scaffolds. In vitro osteogenic mineralization was examined using alkaline phosphatase (ALP) activity and calcium content measurement. Overall, aligned ESNC presented better results than random ESNC. Furthermore, the calcium content was significantly increased in the ESNC functionalized with rhBMP-2, regardless of fiber orientation. As for in vivo bone regeneration, scaffolds were implanted into a rabbit cranial defect. After 3 months, only scaffolds loaded with rhBMP-2 showed some spongy bone formation, where the newly formed bone volume and bone mineral density were significantly higher, nearly fivefold and threefold respectively. These results state that ESCN orientation is considerably less relevant than rhBMP-2 functionalization (Zhang et al. 2019). Zhao et al. prepared lignin-carbohydrate complexes from poplar tree with different ball milling times (48 and 72 h), which were combined with commercial CNF to form tubular biocarriers. CNFs were casted into a polytetrafluoroethylene plate, followed by LCC coating onto the CNF-formed film. The CNF-LCC composite was crimped into a tube (blood vessel-like). Specific surface area of the composite was approximately $3.7 \text{ m}^2 \text{ g}^{-1}$, and the average pore size was nearly 9 nm. The prepared scaffolds presented good pH stability from 4.5 to 9.5. Human coronary artery endothelial cells (HCAECs) were used to assess cell proliferation with CNF-LCC scaffolds in vitro. Overall, CNF-LCC composites that contained an LCC content lower than 60% presented a superior number of cells. Within this range, the number of cells increased with increasing amount of LCC. Above 60% of LCC content, there was no impact on cell proliferation. CNF-LCC composites presented promising scaffold features for biomedical carriers for heart stent tissue since they provide good environment for the growth of human coronary endothelial cells (Zhao et al. 2019).

Wound Dressing

Bacterial infections are responsible for massive worldwide grievous health complications. Due to the loss of antibiotic efficacy, this problem is becoming increasingly a case of concern, as mortality, morbidity, and healthcare costs grow exponentially. Infections that unfold in pressure ulcers, prosthetics, and reconstructive surgeries are particularly worrisome (Hassan et al. 2020). Several emerging applications are under development to cope with this urgent global issue. Poonguzhali et al. prepared biocompatible composites comprising chitosan, poly(vinyl pyrrolidone), and CNC through casting. Thermogravimetric analysis denoted a loss in thermal stability of the composites decreasing proportionally to the increasing content of CNC. Composite thickness, barrier, mechanical, water vapor, and oxygen permeability properties were evaluated. It was determined that the composite was able to maintain an adequate moist environment over a wound. Furthermore, the composite exhibited swelling, blood compatibility (less than 2% of hemolysis), and moderate antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The cytotoxicity of the composite was above the acceptable threshold of 70%, when tested using normal mouse embryonic fibroblast cells (Poonguzhali et al.

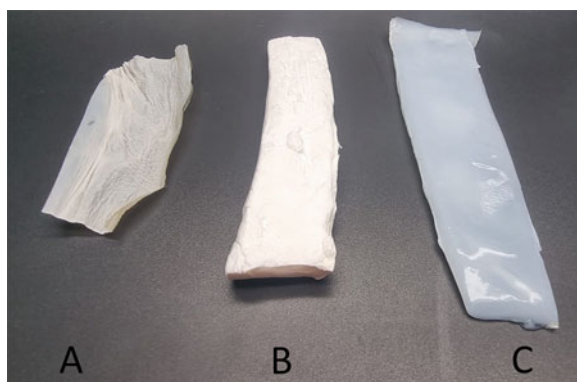
2017). Silva et al. designed a fibrous patch by combining two bio-based nanofibrous polymers, CNF, and lysozyme nanofibers. The singular properties of the mechanically robust CNF and the bioactive lysozyme nanofibers represented an interesting strategy to engineer advanced functional patches with enhanced mechanical performance, UV-light barrier properties, antioxidant, and antimicrobial activities. Biocompatibility, comprising cytotoxicity, cell adhesion and proliferation, and wound healing activity are also key features of these composites. The antioxidant activity of the composites was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging method. The pristine CNF presented nearly negligible antioxidant potential (nearly 7% and 17% after 2 h and 24 h, respectively). CNF does not contain any functional groups capable of behaving as free-radical scavengers, which easily donate hydrogen to reactive free radicals. Pristine lysozyme nanofibers exhibited an elevated radical-scavenging activity (approximately 59% and 85% after 2 h and 24 h, respectively). The combination of CNF with lysozyme nanofibers created a free-standing patch with good antioxidant activity (76–79.5 after 24 h). The DPPH radical-scavenging activity of both patches is comparable to that reported for anti-inflammatory nanocellulose patches loaded with phenolic-based ionic liquids, vitamin B-based ionic liquids, and bromelain. Clearly, this is an important concept when dealing with the excess generation of reactive oxygen species (ROS), which curiously is closely associated with inflammatory processes in wounds. CNF patch per se did not avoid the growth of the pathogenic microorganism. The combination of CNF with lysozyme nanofibers generated patches with antimicrobial activity against *S. aureus*, suggesting that the higher contact availability of the lysozyme nanofibers in the patch results in a higher antimicrobial capacity. Moreover, the developed patches showed to be biocompatible toward L929 fibroblast cells according to ISO 10993-5. The in vitro wound healing assay showed adequate cell migration. The degree of cell adhesion of L929 fibroblast cells on the patches was assessed through SEM for short-term adhesion (up to 72 h) and by live/dead assay for long-term adhesion (up to 21 days). The L929 fibroblast cells' metabolic activity was normal (approximately 100%) for all the investigated patches. However, the SEM micrographs showed that the L929 fibroblast cells adhered differently to the surface of the patches. Poor cell adhesion was observed in pristine CNF patch with no observable confluence. Moreover, after 72 h the cells started to detach. On the other hand, patches encompassing CNF and lysosome nanofibers exhibited a great cell adhesion as a cell monolayer covers throughout the entire surface of the samples (Silva et al. 2020). Yin et al. obtained CNC from commercial microcrystalline cellulose, which was incorporated into the formulation of a gelatin and hyaluronic acid hydrogel. NIH3T3 cell viability and proliferation on the hydrogels was estimated. This study showed that the composite hydrogels lead to an increase in metabolic activity of NIH3T3 cells throughout the incubation period. However, no significant differences in cell viability were observed for the different hydrogel formulations. Furthermore, in live/dead analysis it was possible to observe that most live cells looked like spindles (their healthy morphology) and there were no dead cells in CNC gelatin hyaluronic acid hydrogel. On the other hand, hyaluronic acid and CNC gelatin groups showed few dead cells, whereas the other combinations

presented a cell viability similar to the tissue plate culture. The gelatin, hyaluronic acid, and CNC hydrogels also demonstrated an improvement in mechanical and physicochemical properties, thus making this nontoxic hydrogel a promising applicant for skin wound repair (Yin et al. 2019). Nonleaching antimicrobial surfaces offer great promise for the design of bioactive coatings to be used in medical devices. However, their vast majority are cationic, which usually implies an undesirable toxicity. Thus, Hassan et al. developed an antimicrobial composite that encompassed CNF obtained using a high-pressure fluidizer (raw material: never-dried birch kraft pulp), carboxymethyl cellulose, and through direct functionalization of the surface with dehydroabietic acid derivatives to generate nonleaching anionic surfaces. Interestingly, the wettability of the CNF in comparison to the composites was very distinct. As expected, CNF wettability was high (approximately 30° of contact angle), whereas the composite was nearly hydrophobic (approximately 80°). The composites were able to reduce the concentration of planktonic viable Gram-positive bacteria, including multi-drug-resistant *S. aureus*. The antibacterial surface showed a log reduction from 4 to 5 upon contact. Anti-biofouling properties were also assessed using a clinical isolate *S. aureus* UAMS-1. The composite was able to withstand fouling in a model mimicking the physiological environment in chronic wounds and still exerts a log reduction of 2.5, proving the feasibility of an approach fully based on renewable polymers, as well as a suitable alternative to silver and cationic antimicrobials. Human skin fibroblasts proliferated to a larger extent at the surface of the composite than they did at the surface of CNF. Furthermore, only a minor hemolysis (1.8%) was induced by the best-performing composites. Overall surface is likely to be perceived by bacteria as a complex anionic sugar-based polymeric matrix unevenly branched with the compound, where hydrophilic and hydrophobic regions are not clearly separated but instead have a mosaic distribution of polarity; the films reveal an “imperfect amphipathicity.” The negative surfaces and corresponding mosaic distribution of polarity cannot bind so strongly to positively charged groups in proteins and do not solubilize membranes, and this may help to explain why fibroblasts grow well at its surface and why it does not induce significant hemolysis of red blood cells (Hassan et al. 2020).

Bacterial Nanocellulose

Macroscopically, BNC may be regarded as a 3D or two-dimensional material. If the BNC is not dried, hereby defined as never-dried, then it will display a “wet spongy” macroscopic 3D appearance. The same occurs in the case of freeze-dried BNC, an aerogel with a “frosty” appearance. However, if the BNC is dried (independently if it is dried in an oven or at room temperature) due to the evaporation of the water present within BNC nanofibrils, these tend to collapse by forming additional hydrogen bonds, becoming “paper-like” (Fig. 2). The freedom of the nanofibrils to rearrange is drastically reduced, which considerably reduces stretch ability (Anton-Sales et al. 2021). Furthermore, this event is irreversible despite the swelling degrees achieved by dried BNC (approximately 200%) (Padrão et al. 2016). BNC has been

Fig. 2 (a) Dried BNC;
(b) freeze-dried BNC;
(c) never-dried BNC



used in biomedical applications for at least two decades. In particular, BNC was especially effective per se (when never-dried) as a dressing for burn wounds. BNC offered a sterilized, comfortable, protective, and adequately moist environment for clean burn wounds, which accelerated wound healing (Muangman et al. 2011). Furthermore, never-dried BNC is highly moldable, tightly adapting to the wound site. Never-dried BNC is more stretchable; however, it is less resistant to tear than dried BNC (Anton-Sales et al. 2021). One of the most important BNC limitations is its absence of antimicrobial activity, which greatly limits its application as an active wound dressing and other application prone to infection, such as urinary catheters (Zmejkoski et al. 2021). Therefore, several researchers focused on further enhancing the properties and/or adding functions to this outstanding material. However, in specific applications, BNC per se still possesses highly relevant features. Furthermore, another distinguishing feature of BNC is that its macroscopic shape adjusts to the shape of the area where it is being synthesized by the bacteria (in static culture). Therefore, some studies were focused on the design of BNC conduits to be potentially used as blood vessels or catheters. Some of the recent biomedical applications using BNC are present in Table 2.

Tissue Engineering

Angiogenesis is a key feature of biocompatibility, thus the biocompatibility of the BNC was assessed using shell-less hen's egg model. This model allows the evaluation of the gene activation matrix for regenerative processes on a genetic level, as well as the monitor the embryonic angiogenesis of the area vasculosa. Never-dried BNC and rehydrated freeze-dried BNC did not exhibit any occurrence of hemorrhage, vascular lysis, thrombosis, hemolysis, or death during the entire experiment (24 h). Thus, all BNCs demonstrated the high biocompatibility of BNC and gene-activated matrix since all eggs developed a highly vascularized blood vessel system without changes in the chick area vasculosa. In the same study, the high

Table 2 BNC emerging biomedical applications properties

Bacterium (strain reference)	Functionalized nanocellulose composite	Adequacy	Assays	Cells/animal	Application	References
<i>Komagataeibacter medellinensis</i> (n.d.)	None	In vivo Ex vivo	(a) Ex vivo Hemocompatibility (hemolysis and thrombogenicity); (b) short- and long-term in vivo implantation response (mouse)	(a) Human adult blood (b) Mice	Implantable biomaterial for soft-tissue augmentation or replacement	Osorio et al. (2019a)
<i>K. medellinensis</i> (n.d.)	None	In vitro	Cell adhesion and proliferation (MTT assay), cell morphology (stained with ethidium bromide)	Murine fibroblast NIH3T3 cells	Extracellular matrix (ECM)-like scaffolds for tissue engineering and cell regeneration of soft tissues	Osorio et al. (2019b)
<i>K. medellinensis</i>	BNC modified with NIH3T3 fibroblasts	In vitro	Cell adhesion (AFM), cell proliferation by viability and density [XTT assay and fluorescent staining (RedX donkey-anti-rabbit IgG and DAP)]	Fibroblasts NIH3T3 cells	Extracellular matrix (ECM)-like scaffolds for soft tissue engineering	Osorio et al. (2019c)
n.d.	BNC/CMC/ε-poly-L-lysine (ε-PLL)	In vitro	Cells morphology	Primary human dermal fibroblast cells	Wound dressing	Fürsatz et al. (2018)
<i>Komagataeibacter hansenii</i> (KCTC 10505BP)	Regenerated BNC/gelatin	In vitro In vivo	In vitro cell adhesion and proliferation (FE-SEM analysis), cell viability (MTT assay), immunoblotting (Bio-Rad Protein assay)	Human keratinocytes (HaCaT) cells	Scaffolds for skin regeneration	Khan et al. (2018)

(continued)

Table 2 (continued)

Bacterium (strain reference)	Functionalized nanocellulose composite	Adequacy	Assays	Cells/animal	Application	References
<i>Komagataeibacter sacchari</i> (n.d.)	BNC / poly ([2-(methacryloyloxy)ethyl] trimethylammonium chloride) (PMETAC)	In vitro	Cytotoxicity: MTT assay	Human keratinocyte cell line (HaCaT cells)	Treatment of <i>C. albicans</i> infections	Vilela et al. (2019)
<i>Komagataeibacter xylinus</i> (DSM 14666)	BNC embedded with DNA plasmids	Ex vivo	Biocompatibility testing using Shell-less hen's egg model	n.d.	Biologically active matrix for local delivery of genes	Pötzinger et al. (2019)
<i>Komagataeibacter hansenii</i> (ATCC 23769)	None	In vivo	Implants in brain parenchyma of rats Evaluation through MRI	F98 tumor cells (LGC, Molsheim, France)	Scaffold to trap tumor cells (glioblastoma) after surgical resection	Autier et al. (2019)
<i>K. xylinus</i> (KCCM 40407)	BNC/chitosan	In vitro	Cell adhesion and penetration [histological staining (H&E, F-actin, and DAPI)], cell viability, and proliferation (WST-1 assay)	Human A2780 ovarian cancer cells	3D scaffolds for in vivo diagnosis of ovarian cancer	Ul-Islam et al. (2019)
<i>K. hansenii</i> (ATCC 23769)	BNC/IKVAV (Ile-Lys-Val-Ala-Val) peptide	In vitro	Cytotoxicity (MTS assay), adhesion, and proliferation (L929), morphology (SK-MEL-28)	Murine fibroblast cell line (L929) and human melanoma (SK-MEL-28) cells	Screening of antitumor drugs	Reis et al. (2018)
<i>K. xylinus</i> strain CGMCC (n.d.)	None	In vitro	(a) Blood compatibility (ISO 10993-4:2017) (b) MTT assay Live/dead staining kit	Pig iliac endothelial cells	In vitro blood vessels	Bao et al. (2020)

<i>K. xylinus</i> (ATCC 53582)	None	In vivo	Ultrasound and histological analysis, immunohistochemical staining, analysis of the presence of glycoproteins (periodic acid-Schiff (PAS) staining)	n.d.	Vascular grafts	Weber et al. (2018)
<i>K. xylinus</i> (NCIMB 5346)	None	Ex vivo	Suture test	Pig cornea	Corneal bandage	Anton-Sales et al. (2020)
<i>K. xylinus</i> (X-2)	None	In vitro In vivo	Viability/cytotoxicity (live/dead assay), cell proliferation (CCK-8 assay), and cell morphology	Rabbit primary corneal epithelial and stromal cells	Scaffold for tissue-engineered corneal stroma	Zhang et al. (2020)
<i>K. xylinus</i> (NLQ127)	TEMPO-mediated oxidation (TO-BNC) and maleic acid	In vitro In vivo	(a) Cell viability and live/dead staining assay (calcein-AM/PI dye and microscopy) (b) Osteogenic differentiation (BCIP/NBT Alkaline Phosphatase Color Development Kit) animal experiments	(a) Primary MC3T3-E1 cells (b) Sprague-Dawley rats	Scaffolds for bone regeneration	Wang et al. (2021)
<i>K. xylinus</i>	BNC/collagen	In vitro In vivo	(a) Osteogenic differentiation (b) LIVE/DEAD® Viability/Cytotoxicity Kit (c) Proliferation of UCB-MSCs (d) CCK-8 assay	(a) NIH3T3 cells (b) Human umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs)	Scaffold for bone tissue engineering	Noh et al. (2019)

(continued)

Table 2 (continued)

Bacterium (strain reference)	Functionalized nanocellulose composite	Adequacy	Assays	Cells/animal	Application	References
<i>K. xylinus</i> (BRP2001)	BNC-PHEMA composites	In vitro	Cells viability (AlamarBlue assay) and morphology	Mouse mesenchymal stem cells (rMSCs)	Cartilage, cardiovascular stent, soft tissue engineering, and wound dressing	Hobzova et al. (2018)
<i>K. xylinus</i> (ATCC 53582)	BNC/rabbit bone marrow mesenchymal stem cells (BM-MSCs)	In vitro	Cytotoxicity (production of nitric oxide), toxicity (MTT assay), and phagocytic activity	Mouse (<i>Mus musculus</i>) peritoneum macrophages	Tissue engineering and regenerative medicine	Silva et al. (2018)
<i>K. xylinus</i> (n.d.)	BNC-Fe ₃ O ₄ -HA (magnetite NPs and hydroxyapatite)	In vitro In vivo	(a) Cytotoxicity: MTT assay (b) Biocompatibility (adhesion and spreading)	(a) Fibroblast L929 (b) MC3T3-E1 cells	Scaffold for bone tissue engineering	Torgbo and Sukyai (2019)
<i>K. xylinus</i> (CGMCC)	Chitosan NPs-BNC	In vitro	(a) Cell Counting Kit-8 assay Live/dead assay (b) Subcutaneous implantation	(a) Schwann cells (SCs) (b) Rat model	Potential as scaffolds for nerve conduit	Wei et al. (2021)
n.d.	BNC/waterborne polyurethane (WBPU)	In vitro	Cell viability (PrestoBlue [®]), cell adhesion and proliferation (live/dead assay)	Murine fibroblast L929 cells	n.d.	Urbina et al. (2019)
<i>K. xylinus</i> (ATCC 5352)	BNC/titanium-aluminum niobium (Ti6Al7Nb)	In vitro	Neutral Red (NR) cytotoxicity assay, and fibroblast cells proliferation on BNC (microscopy)	Osteoblast U2-OS and fibroblast L929 cells	Orthopedic implants	Dyda et al. (2018)

<i>K. xylinus</i> (DSM 14666	Cartilage-BNC	In vitro	(a) Viability: fluorescein diacetate/propidium iodide live/dead staining (b) Histology: Hematoxylin/eosin or Safranin O staining (c) Immunohistology: isotype-matched control antibodies staining	(a) Chondrocytes (b) Bovine cartilage (c) Aggrecan, collagens 1 and 2	Cartilage replacement	Horbert et al. (2019)
<i>K. xylinus</i> (NCIMB 5346)	BNC/PP mesh	In vivo	Visual and histological analysis	n.d.	Abdominal wall reinforcement	Anton-Sales et al. (2021)
<i>Medusomyces gisevii</i> Sa-12 symbiotico- culture	None	In vivo	Visual and histological analysis	n.d.	Abdominal wall reinforcement	Zharikov et al. (2018)
<i>K. xylinus</i> (E25)	BNC/PP mesh	In vitro	Cell adhesion (PrestoBlue assay) and degranulation (<i>N</i> -acetyl- β -D-hexosaminidase (HEX) release)	Rat RBL-2H3 basophilic leukemia mast cells	Biomedical applications (reconstructive surgery of soft and hard tissues, e.g., hernioplasty and cranioplasty)	Ludwicka et al. (2019)

n.d., not defined.

loading capabilities of BNC were used to evaluate it as a candidate for carrying DNA. BNC loaded with DNA exhibited good and prolonged transfection efficacy without hindering BNC mechanical properties (Pöttinger et al. 2019). Osorio et al. developed 3D BNC extracellular matrix-like scaffolds for tissue engineering and cell regeneration of soft tissues by cultivation of BNC on a mold containing paraffin microspheres that generate interconnected micropores after leaching. The solvent-exchange method using xylene as dewaxing agent for paraffin microspheres was less time consuming and more suitable for preserving BNC crystal size, resulting in scaffolds with regular porosity and good mechanical behavior. Murine fibroblast NIH3T3 cells could adhere and proliferate, without overcrowding, in scaffolds with small pores and highly interconnected surface area. The scaffolds with extra small pores (approximately 60 μm) presented the highest number of cells on the 16th day (Osorio et al. 2019b). In another study, the microporous 3D BNC surface was populated with fibronectin and collagen type produced by a culture of NIH3T3 fibroblasts. Once confluence reached 90%, the cells were lysed by a mechanical-ionic stress cycle. The presence of collagen type I and fibronectin contributed to a stronger cell adhesion and higher mitochondrial activity (Osorio et al. 2019c). Reis et al. immobilized the pentapeptide Iso-Lys-Val-Ala-Val (IKVAV) in never-dried BNC through carbodiimide chemistry. The envisaged objective was to mimic the microenvironment for human melanoma cells (SKMEL-28). In vitro cytotoxicity tests showed that BNC and BNC-IKVAV did not hinder the metabolism of mouse fibroblast (L929) cells after 7 days of culture. Furthermore, IKVAV improved the adhesion, proliferation, and organization of SKMEL-28 cells, allowing a better in vitro model to screen antitumor drugs (Reis et al. 2018). Autier and coworkers developed a BNC membrane that was used as a scaffold applied to the brain parenchyma after resection of a glioblastoma (Autier et al. 2019). Due to its high flexibility and conformability, the BNC scaffold could be easily introduced into the surgical resection cavity of glioblastoma. Furthermore, the random fibrous nature of BNC is ideal for trapping tumor cells, preventing their movement once attached, thus facilitating targeted treatment and avoiding recurrence. The latter is particularly important since glioblastoma is a highly infiltrative tumor, with a recurrence percentage of 90% within centimeters of the surgical resection cavity. Due to BNC visibility on magnetic resonance imaging, stereotactic radiosurgery may be facilitated during targeted treatment. The trapping capacity of BNC membranes was analyzed by SEM that showed that seeded rat brain glial cells (F98) were able to grow with a spheroid morphology on BNC membranes. In addition, by using transwell migration assay it was verified that F98 cells were exclusively located in the vicinity of inset when the BNC membrane was present. This suggests that these cells permeated at the edge of the insert; however, they were not able to permeate through BNC membrane. Magnetic resonance imaging results showed an edema around the BNC implantation within 24 h. This signal decreased after 21 days and was absent after 3 months. The biocompatibility of BNC was shown by the unaffected brain microenvironment after BNC membrane implantation in the brain parenchyma. Furthermore, after 12 months brain cells were present on the BNC membrane, demonstrating the noncytotoxicity of BNC to normal cells (Autier et al.

2019). Ul-Islam et al. dip-coated BNC in chitosan that was then freeze-dried to obtain a scaffold. The cell–scaffold interaction was analyzed using human ovarian cancer cell lines (A2780). Human ovarian cancer cell lines were found on the surface as well as deeply infiltrated into the scaffold matrix. The incorporation of chitosan greatly improved the probability of enhanced biocompatibility of BNC by promoting cell adhesion, proliferation, and differentiation. Regarding the pristine freeze-dried BNC, cell adhesion was observed. Nevertheless, proliferation was clearly hindered, observed by the presence of larger cell aggregates, when compared to the scaffold. The cell aggregates' sizes on BNC and composite scaffolds were 281 and 133 μm , respectively. Moreover, real-time-polymerase chain reaction (RT-PCR) analysis denoted a decreased mRNA level of Notch receptors in BNC chitosan scaffold, indicating a strong cell–scaffold interaction (Ul-Islam et al. 2019). BNC can be used as a corneal bandage and alternative to amniotic membrane whose availability is highly limited and tissue donor dependent. Essential requirements of ocular surface bandage are their easy application and durable suturing. BNC membrane showed higher suture resistance than amniotic membrane offering good conformability to the eye shape. Furthermore, both materials exhibit low visible light absorbance. In vitro and ex vivo-simulated physiological conditions were performed to assess the durability and stability of the BNC membrane. No significant changes in diameter or thickness as inferred by visual observation and superficial mass measurements were detected (Anton-Sales et al. 2020). Zhang et al. studied the biocompatibility of BNC as a scaffold for corneal stroma replacement using rabbit corneal epithelial and stroma cells. In vitro tests showed a nearly 100% survival rate, good adhesion, and proliferation of the epithelial and stroma cells on BNC. In vivo experiments confirmed the adequate biocompatibility and stability of BNC in rabbit cornea. The optical transparency of BNC was maintained, and no obvious edema or inflammation around the BNC was detected over a period of 90 days (Zhang et al. 2020). Printable scaffolds for bone regeneration were prepared through modification of BNC with (i) TEMPO-mediated oxidation and (ii) maleic acid. After each modification, BNC was hybridized with gelatin (Wang et al. 2021). Both modifications generated a significant change in the nanofiber morphology. In particular, the width was reduced (approximately fivefold), which created a looser fiber network. This proved beneficial for the homogeneous dispersions, consequently improving its printability. The mechanical properties and wetting capability of BNC modified with maleic acid proved superior to the TEMPO-oxidized BNC. The osteogenic marker genes expression of mouse osteoblasts (MC3T3-E1) was also much higher for maleic acid BNC, even though both modified BNC dispersions showed good cell viability for pre-osteoblast. In vivo bone regeneration was assessed using the Sprague–Dawley rat defect model, showing that both BNC composite scaffolds were able to form a larger amount of bone in the skull-defect area. Dispersions demonstrated to be noncytotoxic to MC3T3-E1 cells between 10 and 200 $\mu\text{g/mL}^{-1}$, seemingly promoting cellular viability (114–143%) at 100 $\mu\text{g mL}^{-1}$. According to cell differentiation assays, the BNC dispersion showed slight cytotoxicity after 5 days of incubation, resulting in a decrease in cell viability of 5.4%. In contrast, an obvious increase in cell proliferation was observed in TEMPO-oxidized BNC and

maleic acid BNC groups, with increase in cell viability of 33% and 30%, respectively. Similar results were observed with the staining of live and dead cells. Composite scaffolds for bone tissue were fabricated by Noh and co-workers, containing in their composition BNC and collagen (Noh et al. 2019). Different ratios (1:1, 3:1, 5:1) of BNC:collagen were evaluated. The composite containing the highest BNC content displayed the most organized interconnected porous structure, with improved physical stability and water uptake. Mouse fibroblast and human umbilical cord blood-derived mesenchymal stem cell attachment and growth was favored by the increasing concentration of BNC in the scaffolds. This interesting result was attributed to the higher stability provided by the BNC in comparison to the pristine collagen. Hobzova et al. prepared composites comprising BNC and poly (2-hydroxyethyl methacrylate) composite hydrogels, using UV light to perform in situ radical polymerization. The mechanical properties of the scaffolds containing 10 wt% of BNC were improved, presenting an increase in tensile strength and Young's modulus of 80- and 120-fold, respectively. Moreover, composites were nontoxic, providing a favorable environment for mouse mesenchymal stem cell (rMSC) proliferation. The morphology of the cells was not affected, and the number of viable cells after 72 h of BNC and BNC composites was similar to the tissue plate culture (Hobzova et al. 2018). In another work, Silva et al. studied the in vitro interaction between rabbit bone marrow mesenchymal stem cells and BNC for tissue engineering and regenerative medicine. Cells anchored into the BNC matrix in the first 24 h were fully integrated after 14 days. It was also found the multipotentiality of the cells to differentiate into three lineages, chondrogenic, osteogenic, and adipogenic. Moreover, BNC presented a high cellular viability (approximately 94%) and adequate macrophage activation rate (Silva et al. 2018). An additional, bone tissue-based scaffold was developed by Torgbo and Sukyai, containing magnetite (Fe_3O_4) and hydroxyapatite nanoparticles synthesized by in situ co-precipitation on BNC under ultrasounds (Torgbo and Sukyai 2019). The metabolism of mouse fibroblast L929 cells in contact with the freeze-dried composite scaffold and freeze-dried BNC was evaluated. Results showed a cell viability percentage of nearly 85% for pristine BNC and 74% for the composite. The decrease in cell viability of the scaffold could be the result of the restrictive effects of bonding between phosphate and iron ions. Nonetheless, the scaffold presents great potential in promoting osteoconduction and osteointegration of cells. Furthermore, human fibroblast cell (MC3T3-E1) adhesion on the surface of the scaffold was observed using SEM. On the pristine, BNC exhibited few and sparse cells attached on the surface, likely due to the compact structure, and the absence of osteoconductive and osteoinductive factors. On the contrary, the composite presented a completely covered surface with a healthy and subconfluent monolayer of osteoblast cells. The rougher surface and higher microporosity may have eased the transport and permeation of nutrients and oxygen. Urbina et al. took advantage of the hydrophilic nature of BNC to embed waterborne polyurethane within the never-dried BNC matrix through immersion to obtain a shape memory biomedical application. The obtained composite displayed good water-activated shape memory, flexibility, and transparency. The presence of BNC in these composites contributed to increasing the

shape fixity ability and recovery. The recovery was found to be 92.8% and 33.4% after 3 min for the composite and pristine waterborne polyurethane, respectively. Cytotoxicity tests using murine fibroblasts (L929) showed that both BNC and BNC containing waterborne polyurethane did not hinder cell growth and cell adhesion. Cell viability was significantly higher than the established viability threshold of 70% (Urbina et al. 2019). Dydak et al. immersed titanium–aluminum niobium bone scaffold implants into BNC cell cultures. The implants were fully covered by BNC. Subsequently, the antibiotic gentamycin was incorporated by saturation of the BNC. The BNC-coated scaffolds exhibited a considerably lower cytotoxicity against human osteoblasts (U2-OS) and mouse fibroblasts (L929) cell cultures in comparison to noncoated implants. Furthermore, the incorporated gentamicin exhibited a moderate activity against the proliferation of the bone pathogen *S. aureus*. The zone of inhibition (ZoI) ranged between strong to medium antibacterial activity depending on the gentamycin concentration used for the saturation. However, coated and noncoated did not exhibit striking differences in the generated ZoI. The presence of gentamicin may prevent infections, thus accelerating the recovery process (Dydak et al. 2018). The regenerative capacity of BNC implants was evaluated using a standardized bovine cartilage punch model (Horbert et al. 2019). The host cartilage cylinders were derived from the bovine femoral trochlea using a standardized punch system into which BNC implants were inserted. Progressive colonization was observed for the BNC implants, in concomitance to signs of chondrocyte migration, in particular from the surface of the host cartilage. Such results evidence the high cytocompatibility of the BNC implant in the current in vitro model. The initially cell-free BNC implants presented deposition of the matrix proteins collagen type II and aggrecan throughout culture incubation period. This further proved benign cartilage regeneration. The constant aggrecan release and diminished collagen type II release during the culture suggested that the locally synthesized cartilage-specific matrix proteins seemed to be successfully retained in the BNC implants. Therefore, the lack of in vivo biodegradability makes BNC a sustainable scaffold to support cell ingrowth and subsequent remodeling of the joint cartilage.

Wound Dressing

The absence of hemocompatibility rapidly unfolds into a severe immune response by the host. The ex vivo hemocompatibility and short- and long-term in vivo implantation response to (i) never-dried BNC, (ii) freeze-dried BNC, and (iii) dried BNC was investigated by determining hemolysis and clotting times (Osorio et al. 2019a). The interaction between the erythrocytes and never-dried BNC is reduced by the presence of liquid, reducing the hemolysis percentage to acceptable values. Whereas freeze-dried BNC leads to a quick adsorption of red blood cells, which induces friction, consequently weakening the cell membrane, leading to erythrocytes lysis. Dried BNC did not induce any hemolytic behavior due to its highly packed microstructure, which generates a smooth surface and reduces the friction between red

blood cells and the biomaterial. Thrombogenicity analysis showed complete clot formation in dried and freeze-dried BNC after 15 min. Never-dried BNC did not exhibit clot formation even after 45 min. The high concentration of water present within the never-dried BNC fibrils may reduce the deposition of plasma proteins. In vivo assessment of the immune response to never-dried and freeze-dried BNC demonstrated the absence of signs of discomfort, pain, or disease in the mice throughout the entire experiment. The wound healed after the first week, accompanied by new hair growth. Never-dried and freeze-dried BNC retained their shape, with no sign of dehydration nor purulent inflammation or adverse tissue reactions. Furthermore, all samples showed encapsulation by fibrovascular tissue. In conclusion and comparing dried BNC with never-dried and freeze-dried BNC-based materials, the latter exhibited lower immune response in the first week due to the presence of 3D micropores that allowed the free movement of immune cells and consequent homogeneous distribution. However, for never-dried and freeze-dried BNC the inflammation was resolved after 8 weeks, whereas for dried BNC this event only took 4 weeks. This may be the result of the higher surface area that keeps leukocytes active while they are infiltrating inside BNC. Fürsatz et al. crosslinked low-molecular-weight ϵ -poly-L-Lysine, an antimicrobial peptide with renowned antimicrobial activity, in pristine commercial BNC wound dressings and to carboxymethyl cellulose-functionalized BNC using carbodiimide chemistry. The functionalization had no significant effects on the BNC nanofibrous structure nor on the mechanical properties. ϵ -Poly-L-lysine-functionalized BNC exhibited efficient contact inhibition growth of *Staphylococcus epidermidis* and did not generate cytotoxicity. The primary human dermal fibroblasts cells cultured in contact with all samples, pristine BNC, functionalized BNC with ϵ -poly-L-lysine, and BNC functionalized with CMC and ϵ -poly-L-lysine showed a typical elongated morphology with alignment with the cells on their vicinity (Fürsatz et al. 2018). In another work, Khan et al. developed 3D microporous-regenerated BNC and gelatin scaffolds for the regeneration of skin tissues. Gelatin microspheres were used as porogens and to increase the biocompatibility by eliminating the cytotoxic effects of other porogens. In vitro cytotoxicity tests using human keratinocytes (HaCaT) cells showed good adhesion and proliferation in the matrix, penetrating up to 300 μ m in depth. Furthermore, in vivo tests in the dorsal flank skin of mice using the regenerated BNC and gelatin scaffolds showed complete skin regeneration with a wound closure efficacy of 93% after 2 weeks. In comparison, pristine BNC exhibited only 63% of wound closure efficacy with the same period (Khan et al. 2018). BNC was crosslinked with poly([2-(methacryloyloxy)ethyl] trimethylammonium chloride) (PMETAC) to achieve functional translucent material. This cationic BNC composite exhibited ultraviolet UV-A and UV-B blocking properties. Furthermore, its antifungal properties were evaluated and they exhibited a moderate disinfectant property against *Candida albicans*. The composite cytotoxicity, using human keratinocytes (HaCaT), proved to be dose dependent relatively to its PMETAC content. BNC containing 10 wt% and 40 wt% of crosslinked PMETAC reduced cell viability to $81.4 \pm 2.1\%$ and $72.4 \pm 3.1\%$, respectively. Nevertheless, the accepted threshold for cell viability in biomedical applications is 70%, thus the

BNC-PMETAC composites display a promising potential as a noncytotoxic, nearly transparent, UV-protective, and antifungal application (Vilela et al. 2019). This application is highly promising for see-through diabetic wound dressings.

Indwelling Devices

The potential of BNC conduits as blood vessels was studied by Bao et al. using different designs of bioreactors. As the first approach, the vessels were prepared by placing a single silicone tube inside a hollow glass tube. The cavity between the two was then filled with culture medium containing the bacteria. This method presented lower BNC content, which decreased the mechanical properties. A second strategy was using a similar design, with the sole substitution of the hollow glass tube by another made of silicon, which improved oxygen permeability. The third approach encompassed a glass rod placed inside the hollow silicon tube. These last two bioreactors produced thicker conduits with improved mechanical properties suitable for artificial blood vessel applications. However, still not comparable with expanded polytetrafluoroethylene (ePTFE). Nonetheless, the BNC conduits possessed adequate water permeability, percentage of platelet adhesion, plasma recalcification period, and pig iliac endothelial cells (PIEC) proliferation. Such results suggest better exchange of nutrients, hemocompatibility, and endothelialization after implantation (Bao et al. 2020). Weber et al. designed BNC-layered tubes with reduced wall thickness and smooth surface for small vascular grafts that were tested in vivo by replacing the right carotid artery in sheep. Results showed no delamination in the organism, even after months of implantation. Moreover, histological analysis showed no acute signs of foreign body reaction. However, the tubes were prone to thrombotic occlusion, requiring the administration of antiplatelet drug to lower the occlusion phenomena. Despite the drug therapy, the patency increased from 67% to 80% in a 9-month period (Weber et al. 2018). BNC conduits were biosynthesized in a bioreactor composed of a glass rod and an internal silicon tube. After being collected, purified, and mildly dehydrated using filter paper, chitosan was injected into its internal cavity. Chitosan nanoparticles were synthesized within the BNC fibrils via ionic gelation method. The mechanical properties and matrix structure of never-dried BNC were retained. Furthermore, composites depicted strong decontaminant antibacterial activity against *S. aureus* and *E. coli*. Furthermore, the composites were able to promote the adhesion and proliferation of Schwann cells and demonstrated good biocompatibility after subcutaneous implantation in rats. In fact, all tested samples exhibited absence of cytotoxicity and biocompatibility, including pristine BNC. Despite the occurrence of Schwann cell proliferation after 5-day incubation on all surfaces, the number of cells on the composite containing the highest concentration of chitosan nanoparticles was significantly less, which indicates that the material was not as conducive to cell proliferation in comparison to all the other tested samples. These results suggest that excessive chitosan nanoparticle concentrations have a cytolytic effect. Accordingly, the obtained results suggest that an adequate concentration of chitosan nanoparticles could prevent the potential

cytolytic and cell inhibitory effect due to excessive amounts of cationic chitosan nanoparticles, while maintaining the efficient antibacterial activities. Histological staining of pristine BNC and an intermediate concentration of chitosan nanoparticles (0.5%) confirmed the good biocompatibility of these materials. The staining demonstrated the absence of inflammatory response after implantation with fibroblasts attached to the surfaces of BNC and the composite. Masson staining showed a large number of collagen fibers formed around the material, demonstrating tissue integration (Wei et al. 2021). BNC's potential as an indwelling device, namely, as reinforcement meshes, was also analyzed. In soft-tissue repair materials, it is important to have implants with antiadhesive characteristics to minimize complications caused by intraperitoneal adhesions. Anton-Sales et al. developed an antiadherent reinforcement material to support hernia soft tissues by combining BNC with standard polypropylene (PP) meshes. First, the authors confirmed the suitability of double-layer BNC for abdominal wall reinforcement regarding mechanical properties (tear strength $>16 \text{ N cm}^{-1}$). Then, a hybrid material was developed by integrating PP meshes between the BNC layers and the antiadhesion properties of BNC with an in vivo rabbit model following the implantation process were evaluated. The results showed a general good integration to the abdominal wall. Histological analysis showed an inflammatory response in the peri-implanted area presenting fibroplasia and infiltration of diverse cell types, such as macrophages, lymphocytes, and heterophilic granulocytes, among others. Nevertheless, the antiadherent properties were clearly proved, underscoring its applicability in hernia repair solutions. The only identified issue was its suboptimal mechanical behavior, which may be surpassed by stacking dried BNC membranes or by the development of novel composites (Anton-Sales et al. 2021). A similar approach was performed by Ludwicka et al. The obtained PP mesh coated with never-dried BNC did not negatively influence the mechanical properties of the commercial PP mesh, and it envisioned less immunogenic reaction toward the composite by lowering mast cells adhesion and degranulation. PP mesh, pristine never-dried BNC, and the composite exhibited significantly less rat basophilic leukemia mast cells (RBL-2H3) cell adhesion in comparison to TCP (roughly threefold less). Regarding mastocyte degranulation level (percentage of N-acetyl- β -d-hexosaminidase (HEX)), it was reduced from approximately 32% in PP mesh to 19% in the PP coated with BNC composite. However, the degranulation in pristine BNC was slightly lower (Ludwicka et al. 2019). Therefore, the role of BNC in suppressing mast cell degranulation is clear. Mast cells are present in all smooth muscles; therefore, they will be in contact with all indwelling devices. Mast cells degranulate once they are activated, releasing a plethora of inflammatory mediators present in their up to 200 granules in each cell. These mediators are able to promptly unfold into a violent immunological response (Krystel-Whittemore et al. 2016). In the study carried out by Zharikov et al., never-dried BNC membranes were implanted in the abdominal wall of dogs to study its efficiency in hernia repair. The antiadhesive behavior of BNC was compared with a commercial PP mesh. After 14 days of implantation, a capsule of loose connective tissue around the BNC was observed, exhibiting good fixation to the anterior abdominal wall, and negligible adhesions between the BNC

membrane and the underlying tissues. Although some moderate inflammatory infiltration was occurring due to the accumulation of cell elements at the interface of BNC and the abdominal wall tissues. After 2 months, the host formed novel and stable connective tissue and angiogenesis were observed. Furthermore, negligible intraperitoneal adhesions between the intestinal loops and the BNC membrane were detected. Within this period, the commercial PP mesh-based grafts where coarser and scar structures were observed denoting the superior biocompatibility of BNC (Zharikov et al. 2018).

Conclusion

Nanocellulose clearly exhibits an enormous potential for the development of innovative and highly efficient biomedical applications. The fields covered by nanocellulose applications encompass scaffolds for tissue regeneration, enwalling devices, drug carriers, antimicrobial patches, among others. Interestingly, these properties are intertwined in a single device through modification and composite development. This is due to remarkable eclectic properties of nanocellulose, which virtually allow the stacking of functions and properties within a single use. CNC and CNF, the most emerging technologies, are focused on their use in producing ESNC, WSNC, and as printable applications. Printable applications allow the tailoring of macroscopic structures that possess the advantageous nanoproperties of CNC and CNF. BNCs' plethora of biomedical applications has highlighted that, despite all functionalizations and modifications, pristine BNC per se sometimes denotes an unmatched biocompatibility. In particular, never-dried BNC seems to have the most biocompatibility and hemocompatibility features in comparison to all nanocelluloses. Its use as a coating of materials, generated during bacteria culture, is a promising strategy for the development of superior biomedical applications.

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Sensing and Biosensing Applications of Nanocellulose

35

Meenu Eldhose, Roshny Roy, Cincy George, and Alex Joseph

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M. Eldhose · R. Roy · C. George · A. Joseph (✉)
Department of Chemistry, Newman College, Thodupuzha, India
e-mail: alex.joseph@newmancollege.ac.in

Abstract

Cellulose in its nanostructuric form gained great attention among many researchers owing to the natural abundance, high aspect ratio, ease of surface functionalization, biocompatibility, and better mechanical and chemical properties. NC is recognized for its remarkable inherent properties like chemical inertness, amphilic nature, mechanical strength, high stiffness, and low density extends its application toward many electrochemical fields. Nanocellulose is used as a structural component in many electrochemical sensors due to the large number of hydroxyl groups on the surface, which can be structurally modify in order to tune the chemical and physical properties suitable for sensor applications. This chapter describes the features of nanocellulose, methods for structural modification, application as a structural component, etc., relevant to sensing applications. A detailed description on use of NC for application like electrochemical sensing including biosensors, molecular sensors, and gas sensors are included.

Keywords

Nanocellulose · Surface functionalization · Electrochemical sensing · Biosensing

Introduction

Nanocellulose (NC) is one of the most fascinating biomaterials present abundantly in nature. The main natural resources of NC are wood, blast fibers, marine animals, algae, bacteria, and invertebrates, extracted either by chemical, mechanical, or enzymatic methods. It is considered as a green sustainable substitute for the existing ever depleting petroleum-based material, currently prevailed in commercial and industrial applications due to its natural abundance and biodegradable nature. Other contributing factors of NC are the chemical inertness, excellent stiffness, low density, and plenty of surface functional groups present on the surface make NC a versatile material for many research applications. Consequently, NC is used in a variety of fields such as biomedical applications, energy sector, electrochemical sensors, and in reinforced plastics. Altogether NC is considered to be a futuristic material with lots of research potential and is in the pipeline in order to be implemented as a green sustainable material.

One of the interesting properties of NC is the presence of highly susceptible hydroxy groups present on the surface facilitating the tuning of physical and chemical properties. Also, the high aspect ratio (surface to volume ratio) of NC increases the scope of this material to be used as a substitute for many other synthetic nano-substrates. The process of surface functionalization is now turned out to be one of the best methods to tune the physical electronic and chemical properties to fit into desirable applications like sensors (Nguyen et al. 2019), biomedical applications (Jorfi and Foster 2015), drug delivery (Hasan et al. 2020),

etc. NC has hydroxyl groups enriched on its surface, making it a hydrophilic surface. Indeed this helps the researchers to tune the properties of NC through chemical (Habibi 2014), physical (Islam et al. 2013), and biological modifications (Heise et al. 2021) with suitable molecular receptors and catalysts. The role of selected functional moieties used to modify are transduction with specific analyte molecules. The surface modification can be done either by post functionalization or in situ functionalization. Also, the surface functionalization is a state-of-the-art technique to endow surface with appropriate charges like positive or negative charge so as to facilitate the physical absorption of foreign reactive molecules. Surface functionalization not only helps tuning of structural and chemical properties but also provides a uniform distribution of surface active agents, maximizing the analyte signal transduction property. Due to these properties, NC is considered to be the most suitable natural material for electrochemical sensors as it is biodegradable, renewable, and causes no incompatibility issues with biological systems.

Nanocellulose as a Structural Component in Electrochemical Sensors

In addition to plenty of hydroxyl groups on its surface, nanocellulose has high surface to volume ratio, which makes nanocellulose as a suitable material for electrochemical applications. In addition to this, the natural abundance and biodegradability increases the scope of this material to use in many electronic applications.

Surface functionalization is one of the key strategies to tune the electrochemical property of any desirable material for electrochemical applications. The surface hydroxyl groups present on NC is the best to subject to either post chemical functionalization, biological, or physical modifications using appropriate molecular receptors (Du et al. 2017). Many scientists have worked toward the development of NC as a base material for construction of electrochemical molecular and biosensors. The main component of an electrochemical sensor is a modified electrode as the working electrode, which interacts with the analyte, transducing the signal to a read out. The electrode architecture of a sensor consists of a series of layers called electrode base, active material, and receptors built using appropriate materials. A pictorial representation of the working electrode on the hierarchical pattern is represented in Fig. 1.

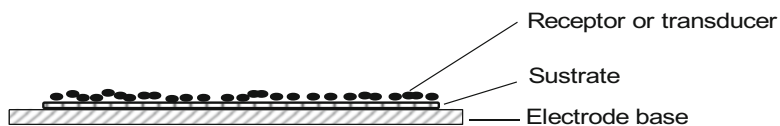


Fig. 1 Pictorial representation of electrode architecture of an electrochemical sensor

NC has been used as an active material for the preparation of electrode for electrochemical sensing. The primary reactive site is the hydroxyl group present on the surface and this will help to form a uniform distribution of the functional receptors. This is unlike the other nanostructures like carbon nanotubes or graphene used in many electrochemical sensors which possess the reactive functional groups on the periphery rather than in the middle. Another advantage of surface functionalization is the increase in the dispersion ability of the functionalized nanocellulose in hydrophobic media, enhancing the signal transduction property. Also, it reported to significantly improve the tensile and share properties of the composites (Lu et al. 2019). The surface of nanocellulose can be modified using many chemical receptors. These methods of modification involve sulfonation (Xu et al. 2020), covalent chemical modification, and polymer grafting ester modification, silylation, substitution with nucleophiles, carbamation, and oxidation (Horta-Velázquez and Morales-Narváez 2022). Another advantage of using nanocellulose is its ability to prevent aggregation or agglomeration as it is reported by Park et al (2013). Graphene is one of the widely used components in electrochemical sensors subjected to aggregation in hydrophobic media. Nanocellulose is used as a dispersion media to prevent agglomeration to stay graphene apart so as to enhance the transduction ability and signal strength. Nanocellulose can used to form a transducer surface in different forms, composites, membranes and films, form convinces the transduction ability (Edwards et al. 2016a; Ling et al. 2019). This will ensure a high catalyst loading, endow superior sensitivity, and selectivity. Also the formation of porous structures like hydrogel, aerogel, or sponge provides an additional improvement in surface area imparting excellent catalytic activity toward analytes (Bonné et al. 1985). Depending upon the source of the nanocellulose, the quality of the nanocellulose also varies. There are reports telling nanocellulose extracted from bacteria possess a 3D network structure with high degree of polymerization and crystallinity (Korhonen et al. 2011). This structure possesses a 3D network of cellulose fiber and imparts additional improvement in surface area in comparison to that of any other nanocellulose. This 3D structure caters as a structural material, as a carrier for a high amount of nanoparticles than any other nanocellulose synthesized from other conditions.

Methods of Surface Modification

The research of functional nanostructured materials has been a renowned subject of study because they have significantly improved features and qualities, allowing these high-value-added substrates to be used in materials science. The NC has a hydrophilic surface which prevents the usage of it in hydrophilic or polar media. The surface of NC which is hydrophilic in nature owing to the presence of hydroxyl (-OH) groups can be easily modified by physical, chemical, and biological processes. The surface modification of NC would increase all the properties such as tensile strength and tensile modulus which makes it a competent and innovative material for variety of applications.

Chemical Modification of NC

The surface modification can be achieved during preparation or post-preparation. There are many methods used for the functionalization of NC, Lin et al. (2012) stated surface modification of polysaccharide nanocrystals can result in polymeric matrices with improved thermal and mechanical capabilities. The cellulose nanofibers are highly reactive and can be readily functionalized using the hydroxyl groups present. Sulfonation, esterification, etherification, carbamination, polymer grafting, oxidation, nucleophilic substitution, TEMPO mediated oxidation, urethanization, amidation, silylation, and click chemistry are some of the chemical processes which can be employed to modify the surface of cellulose nanocrystals. A schematic representation of the chemical functionalization route with reaction conditions is represented (Fig. 2).

Pendant surface hydroxyl groups, typically the primary alcohol group (CH_2OH), are used to chemically modify the surface of cellulose nanoparticles. On nanocellulosic materials, various chemical modification strategies were used to (1) enhance the effectiveness of the separation process and (2) alter hydrophobicity of the surface, which enhances the dispersibility and compatibility of nanocelluloses in certain solvents. The partial functionalization of the surface of nanocellulose produced by sulfuric and phosphoric acid hydrolysis results in sulfate or phosphate esters. The charge which is required for aqueous dispersion and isolation operations are

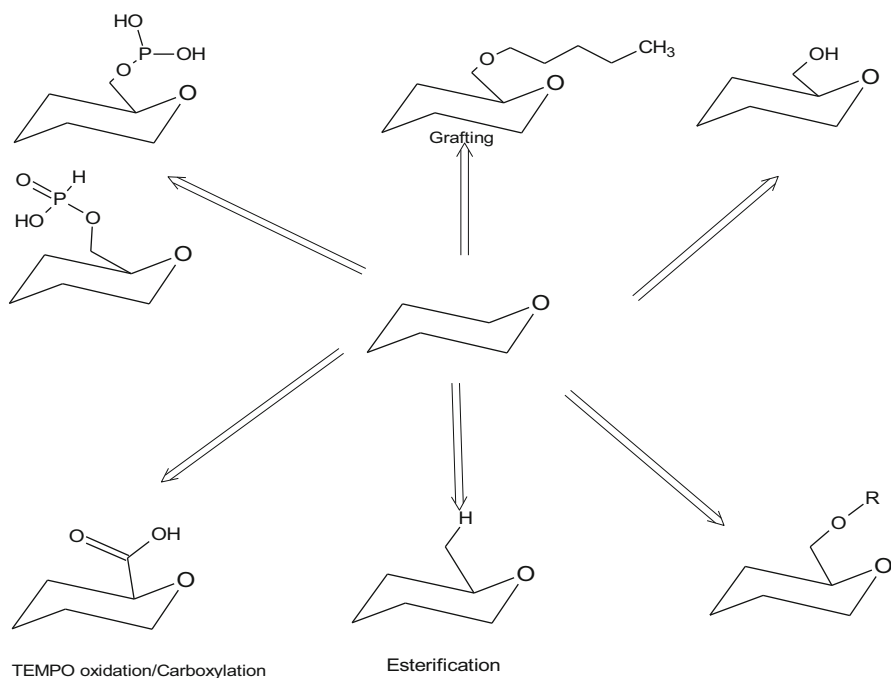


Fig. 2 Showing the ways of chemical modification

introduced in this way. Phosphorylation, carboxymethylation, oxidation, and sulfonation processes have all been used to introduce ionic charge to the cellulose surface.

Lu et al. (2019) studied hydroxyapatite modified nanocellulose with polylactic acid (PLA) as in the matrix in order to overcome the inbuilt hydrophobicity of polylactic acid. According to the findings, the hydroxyapatite used modified nanocellulose which shows enhancement in the mechanical properties of poly lactic acid-based nanocomposite films by causing strong hydrogen bonding interactions at the surface, resulting in comparatively good dispersion in the polylactic acid composite. As a result of the surface modification, the tensile strength, Young's modulus, and thermal stability of the nanocomposite are increased which indicate that the hydroxyapatite-modified nanocellulose is a good PLA reinforcing material. Along with the chemical modifications there are certain physical methods to modify the surface of nanocellulose. Electric discharge or plasma treatment, ultrasonic treatment, irradiation (Pakharenko et al. 2016), and surface fibrillation (Wakabayashi et al. 2020) are some of the physical surface functionalization procedures employed by the researchers (Fig. 3).

For a variety of applications, click chemistry allows the synthesis of (bio)chemically active cellulose surfaces (Benkaddour et al. 2013). In addition, an interesting research shows how to make 2D crystalline cellulose oligomers containing surface-reactive azide groups utilizing click chemistry and an enzymatic reaction. The enzymatic method is helpful for the long-term production of cellulose oligomers in aqueous as well as in mild conditions, and is commonly accomplished by the process of glycosylation involving cellulose and cellodextrin phosphorylase. The merits of the reaction includes high yield and good functionalization.

Biochemical Modification of NC

Enzymes are also used for the surface modification of nanocellulose, and it is of two types: direct and indirect methods. Two of the most prominent types of enzymes are hydrolases and oxidoreductases. Examples for the hydrolase (Afrin and Karim 2017)

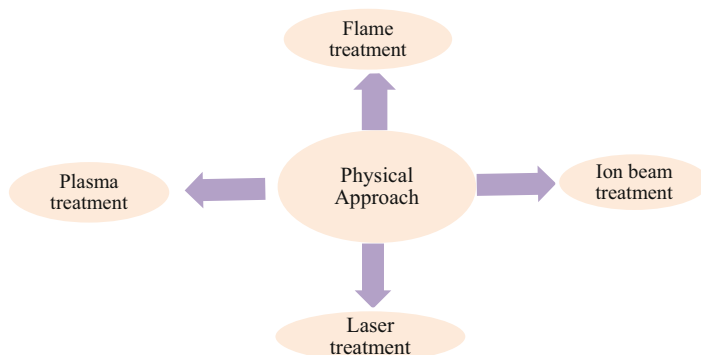
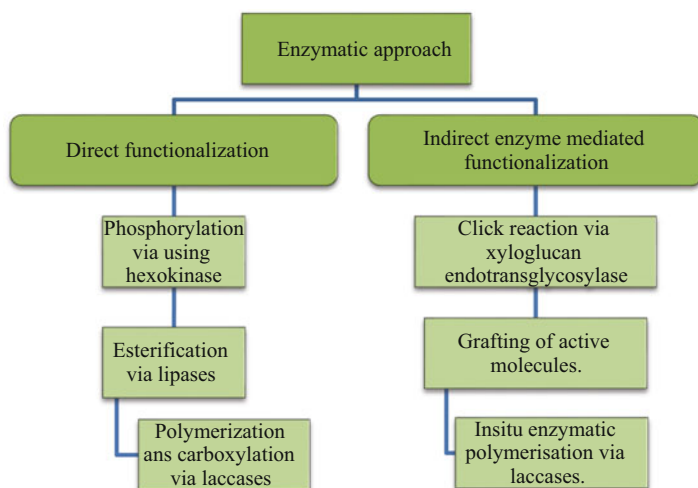


Fig. 3 Representative diagram showing the physical methods of modification of NC surface

enzymes includes glycosidases, lipases, and proteases that are commonly utilized, whereas oxidoreductases (Karim et al. 2017) include laccase, peroxidase, and tyrosinase that are commonly used. The enzymatically functionalized nanocellulosic materials have a considerable advantage over chemically functionalized materials since they prevent the toxicity of the transformed product so they can be used effectively in biomedical applications. Lipases, the most extensively employed class of enzymes for catalytic reactions, have been effectively exploited for stereoselective and regioselective acetylation (Mahapatro et al. 2006) of a variety of compounds, including cellulose. The high specificity of enzymes will soft the reaction conditions, and provide a non-destructive transformations of polymers. The enzyme modification of the polymer is safer and more advantageous than chemical techniques. Enzyme retting, for example, is able to solve the issues connected with dew and water retting since the fibers are treated in a carefully controlled environment and the fibers thus generated have the same quality as water-retted fibers and without large amounts of fermentation waste.



Some of the reports on enzymatic functionalization tells that nanocellulose was modified by oxidation with the help of the dehydrogenase enzyme (Lin and Dufresne 2014). Nanocomposites of nanocellulose/polypyrrole is used and due to the huge effective surface area of the composites generate large catalytic oxidation currents and enable charge storage. Again the enzyme galactose oxidase is used to perform direct oxidation of nanocellulose (Liu and Chen 2016) followed by selective carboxylation, yielding galacturonic acid derivatives with high conversion and yield.

Enzymatic polymerization of synthetic and natural materials onto substrate is environmentally acceptable as the synthetic process demonstrate the value of “green polymer chemistry.” It also benefits milder reaction conditions such as temperature, pressure, and pH without the usage of harmful chemicals. Enzymatic grafting onto NC is practiced to graft active compounds onto the surface of nanocelluloses. The process is divided into two categories: grafting onto it and grafting from. The

grafting-onto approach includes attaching synthesized chains to reactive cellulose hydroxyl end groups. For example, Akhlaghi and Najafpour-Darzi (2020), looked at the susceptibility of the corresponding bacterial nanocellulose fibers as a reinforcement agent in cement composites. It is observed that the resultant BNCs cement mortars have enhanced mechanical properties. Alternatively, the grafting techniques are used to boost the concentration of the active material on the surface and to ensure its stability for various applications. The interactions arise between nanocellulose and active molecule surface are physical, chemical or covalent interactions, are all necessary to immobilize active molecules onto the nano cellulose surface. The most serious issue with chemical grafting of active molecules particularly bioactive compounds is the uncontrolled linkage between nanocellulose and active molecules, which can result in protein attachment at a position that changes its structure, stability, or bioactivity. This will alter the course of the selectivity of the corresponding enzymes toward the substrate. Therefor an efficient and orthogonal strategy to overcome these limitations during transformation is to use enzyme-mediated grafting/immobilization. It also doesn't require any organic solvents and occurs under physiological settings. It could be used on a wide range of functional proteins because it is site-directed and regiospecific. Additionally, a chemo-enzymatic method for, for instance, grafting onto the surface of protein immobilization was created using the enzyme transpeptidase (Parikka et al. 2012), from Gram-positive bacteria, which is employed to covalently attach proteins to peptidoglycan layers. This enzyme requires two brief recognition sequences for ligation and creates a native amide bond. Enzymes are used in the production process to further functionalize nanocellulose, which makes it more environmentally friendly and widely accepted in both small-scale and industrial settings. They also appear to be less expensive than chemical processes due to their high enzymatic hydrolysis efficiency, which boosts manufacturing yield, low energy consumption, and relatively high power of penetration, which refers to their capacity to adsorb or cover the interior surface of biomass resources. As a result, using an enzymatic technique for the creation and modification of nanocellulose has numerous advantages over using a chemical approach.

Source of cellulose	Nano-cellulose type	Synthesis method of nano cellulose	Strategies for the surface modification	Properties enhanced	Application
Dry eucalyptus lap wood pulp	CNC	H ₂ SO ₄ hydrolysis	Using methylated canola oil fatty acids for transesterification ester	Improves higher thermal stability and hydrophobicity	Used to create hydrophobic coatings and nanocomposites with hydrophobic polymer as reinforcement (Wei et al. 2017)
From softwood pulp	CNF	Oxidation mediated by TEMPO method	Cetyltrimethylammonium grafting as a surfactant and bromide	The polymer has improved thermostability and hydrophobicity	It helps to improve the redispersibility of the TEMPO-oxidized nanocellulosic fibers in N, N-dimethylformamide (Qu et al. 2019)

(continued)

Source of cellulose	Nano-cellulose type	Synthesis method of nano cellulose	Strategies for the surface modification	Properties enhanced	Application
Sugarcane bagasse	CNC	H ₂ SO ₄ hydrolysis	The functionalization of CNC was done using adipic acid	Thermodynamic wetting and improved dispersion	Used as a reinforcement for the hydrophobic polymer matrices (Ferreira et al. 2018)
Softwood sulfite fibers	CNF	Grinding	Followed by sonication in the presence of lactic acid	Enhanced mechanical properties and the water draining	Applied as a performance-enhancement additive in traditional papermaking industry (Sethi et al. 2018)
Blue agave bagasse (A tequilana)	CNF	Homogenization is done at high pressure	Using 3-aminopropyl triethoxysilane to silane	Mechanical and hydrophobic properties were improved	Adding agent to PLA to create reinforced composites (Robles et al. 2015)
Blue agave bagasse (A tequilana)	CNC	H ₂ SO ₄ hydrolysis	Esterification with dodecanoyl chloride	The hydrophobicity and thermal stability enhances	Used as additives to strength various composites
Aspen Kraft pulp	CNC	H ₂ SO ₄ hydrolysis	Oxidizing CNC with sodium periodate and then immobilizing it covalently with black wattle tannin	Increased metal adsorption capability and improved regeneration and reusability	It can be used as novel nanocomposites to eliminate contaminants from industrial effluents (Xu et al. 2017)

Another aspect is NC can act as a template for the fabrication of sensing materials. The advantage of NC over any other conventional template molecule is its ability to cast its chiral nematic liquid crystal pattern to inorganic solids for a variety of photonic applications. In this method, NC is used as a template for inducing its structural features into matrix by post incineration.

Nanocellulose as a Structural Component in Sensors

NC is very unique toward construction of sensors owing to the superior mechanical, optical, thermal, and structural properties. The structural aspects like high surface area, high aspect ratio, and large specific properties are beneficial for construction of sensor devices. Also the functional groups on surface is succceptable for receptor functionalization improves the possibilities of using NC for variety of sensor application. Surface functionalization is a way to tune the physical and chemical properties in such a way that the analyte specifically interact with the NC substrate thereby improving the sensitivity. There are many kinds of electrochemical sensors, some of them are discussed with few examples.

Biosensor

A biosensor is an integrated system of transducer-receptor which has a physiochemical detector for sensing biological analytes. The immobilized matrix of biosensors has a substantial impact on sensing performance which helps in biorecognition units like enzymes, antibodies, cell receptors, or organelles. These sensors' tiny size, low cost, and ease of use make them perfect point-of-care diagnostic instruments. The transducer surface of the biosensor surface has a major impact on the sensing and detection limit of the biosensor. Based on the structural resilience, compatibility, and hydrophilicity of the nanocellulose, it is an ideal material to use as a structural component in biosensor.

Glucose Sensor

The need for glucose control in diabetic patients has been long recognized. This monitoring helps to reduce the risk of complications of acute diabetes, nephropathy, neuropathy, and microvascular complication of retinopathy. The 1-D nanocellulose-based sensor can be used to enhance glucose-sensing due to its active surface properties. Because of its high surface area, unique particle size, and pore shape, nanocellulose is an excellent organic support material for enzyme immobilization. The enzyme immobilization was done using glucose oxidase, peroxidase, and o-dianisidine (Cha et al. 2012) makes up the sensing system. The detection of glucose in serum and cell culture can be done by visual and nonenzymatic assay. Uddin et al. (2019) demonstrate the use of nanocellulose for enzyme immobilization in glucose detection in the disposable microfluidic sensor. In the microfluidic sensor, the nanocellulose create a storage for glucose oxidase and also act as microfluidic channel. The NC is also used as a support for non-enzymatic sensors. Liu et al. (2016) synthesized silver nanoparticles (AgNPs) with CNC as a substrate. The obtained AgNPs-CNCs are superior to the commercial ones, as they have lower minimal inhibitory concentration in the presence of CNC due to the dispersion of the nanoparticles. The following table describes the sensing techniques employed for sensing glucose along with its LOD, specificity, and the role of NC.

Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit	Reference
CNC	Colorimetric	Substrate/dispersant	0.116×10^{-6} M	Wang et al. (2016)
CNF	Colorimetric	Substrate	100×10^{-6} M	Liu et al. (2011)
CNC	Calorimetric	Substrate	1.5 mM	Neubauerova et al. (2020)

Cholesterol Sensor

Cholesterol is the most common sterol synthesized by most human cells; works as a precursor for the manufacture of steroid hormones, vitamin D, and bile acids; and is a vital component of cell membranes. High levels of cholesterol can lead to cardiovascular illnesses, heart attacks, strokes, peripheral arterial disease, and type 2 diabetes. The 1-D nanocellulose supported sensor is a self-powered novel cholesterol sensor. CNC is the perfect substance for enzyme loading of cholesterol oxidase (ChOx) and fabricating chloesterol sensor. Nanocellulose chemically modified with zinc oxide nanoparticles along with silylated graphene oxide and cholesterol (Anirudhan and Deepa 2018) shows good conducting characteristics. Limit of detection (LOD) values were 7.4 mol/L over a detection range of 5.18 mol/L to 25.9 mol/L. The ideal pH and response time were discovered to be 7.4 and 10 min, respectively. With a LOD value of 0.0986 mmol/L, DPV analysis showed strong linearity throughout the 0.6475–10.360 mmol/L range for cholesterol sensing. Again, it is reported that the in situ generated polyaniline (PANI) on CNC by the oxidative polymerization process is a suitable material for enzyme loading and improved conductivity. Abdi et al. (2019) also synthesized a cholesterol biosensor with polyaniline/CNC/ionic liquid to modify screen-printed electrodes. The cholesterol biosensor was fabricated by immobilizing cholesterol oxidase (ChOx) on the modified electrode. Some of the recent results are summarized in the following table.

Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit	Reference
CNC	Voltametric	Matrix/carrier	7.4×10^{-6} m	Anirudhan and Deepa (2018)
CNC	Voltametric	Matrix	0.48×10^{-6} m	Abdi et al. (2019)
CMNC	Voltametric	Matrix/carrier	7.43 μ mol/L	Anirudhan and Deepa (2018)

Human Neutrophil Elastase (HNE) Sensor

Studying the changes in the wound nature can enhance patient care pathways and clinical results considerably. The chronic wound biofluid contains proteolytic enzymes, chemokines, growth factors, electrolytes, and cytokines. Proteolytic enzymes, such as serine proteases (HNE), are suitable biomarkers because they reflect wound healing progress. The HNE sensor is a promising biomarker for inflammatory disorders. Nanocellulose of dimensions 1-D, 2-D, and aerogels performs as a substrate and matrix for the transducer in HNE sensor. The one-dimensional CNC is used to make a colorimetric sensor (Ligi et al. 2016) where the cotton-derived CNC is conjugated with tripeptide (Ligi et al. 2016). This glycine esterified CNC is covalently attached to HNE tripeptide *n*-succinyl-alanine–alanine-

valine-paranitroanilide (Suc-Ala-Ala-Val-pNA) shows higher HNE activity than CNC/peptide conjugate is supported on paper. Ling et al. (2019) produced 1D-nano-cellulose-based colorimetric biosensors, cotton derived nanocellulose to device peptide-cellulose conjugate HNE protease sensor. The higher degree of sensitivity and substitution of CNC compared to 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-oxidized wood cellulose nanofibrils, which results in larger crystalline size and higher crystallinity gives an advantage of increased accessibility for peptide interaction and tetrapeptide immobilization on cellulose crystals.

Fontenot et al. (2017) developed a biosensor for the detection of HNE in chronic wound fluid using CNC and fluorescent elastase tripeptide or tetrapeptide. Due to the CNCs' great specific surface area, which enables for the loading of significant amounts of elastase peptide, their sensitivity can reach 0.5 U/mL. HNE et al. (Fontenot et al. 2017) developed a fluorescence sensor using peptide coupled cellulose and nanocellulose. Compared to matrices of cellulose, the nanocellulose has a higher sensitivity than their peers in detecting HNE, due to the increased specific surface area of CNC films and therefore CNC peptide products. The 3D nano cellulose structures are employed with an enhanced sensitivity. Edwards et al. reported the development of a protease detection sensor using aerogels made up of peptide and nano cellulosic. The C6 hydroxyl groups in the nano cellulosic aerogels were first esterified with a glycidyl-fluorenyl methyloxycarbonyl group, followed by the deprotection and coupling of tripeptides with immobilized glycine. The results are promising with a detection limit of 0.13 units per mL. Some of the similar examples are listed in the following table.

Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit U mL ⁻¹	Reference
1D-CNC	Colorimetry	Matrix	0.05	Edwards et al. (2013)
1DCNC	Colorimetry	Substrate	0.005	Ling et al. (2019)
1D-CNC	Fluorometry	Matrix	0.015	Condon and French (2019)
2D-CNC	Fluorometry	Matrix	0.015	Fontenot et al. (2017)
Aerogels	Fluorometry	Matrix	0.13	Fontenot et al. (2016)

Human Serum Albumin (HAS) Sensor

Human serum albumin is one of the essential macromolecule in the plasma of human blood. Abnormal HSA levels have been linked to multiple myeloma, nephropathy, coronary heart disease, diabetics, nephropathy, liver cirrhosis, and other conditions. As a result, accurate and quantitative detection of HAS in biological science, molecular biology, clinical medicine, and other domains has enormous research and practical value. Naghdi et al. (2019) synthesized curcumin embedded bacterial cellulose nanopaper (CEBC) by simply soaking bacterial cellulose (BC) nanopaper in curcumin solution and drying it at 100 °C. The hydrophobic wall is created by printing a toner layer onto the dried film of CEBC which is used as nano paper-based

analytical device (NAD)-CEBC. The BC nano paper is a proper platform owing to its transparency, flexible, porous, biodegradable, and printable for the analytical device. The key strategy is, the various inhibitory effect of curcumin degradation by HAS in alkaline solution results in color change and this change in color can be monitored spectroscopically or visually. The results, which were compared to those from the industry-standard Bromocresol Green (BCG) Albumin Assay Kit, were consistent.

Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit	Reference
CNC	Colorimetric	Matrix	5×10^{-6} M	Edwards et al. (2016b)
CEBC	Voltametric	Matrix	200 mM	Naghdi et al. (2019)

Purines (Adenine and Guanine) Sensor

The essential components of both ribonucleic acid (RNA) and deoxyribonucleic acid are guanine and adenine (DNA). To understand the DNA sequence, protein metabolism in cells, oxidative damage, and hybridization sensitivity, and selective detection of adenine and guanine are required. These nitrogen bases hinder the direct electron transfer as well as induce irreversible adsorption on standard electrode surface tendering low repeatability and sensitivity, making it difficult to acquire enough sensing signals. In an effort to overcome these restrictions and provide things like a wide potential window, strong electrocatalytic activity, and antifouling property, many materials have been employed to improve electrodes. For fabricating a thin film for electrochemical activity, a new composite employing NC and SWCNH with the intention of creating a thin film for electrochemical uses. It is anticipated that this substance will have highly desirable antifouling and catalytic qualities for electroanalysis. Ortolani et al. (2019) proposed an electrochemical sensor for sensing guanine and adenine based on CNC and single-walled carbon nanotubes (SWCNH).

Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit	Reference
CNC	Amperometry	Matrix	0.17×10^{-6} M	Naghdi et al. (2019)
NC	Voltammetry	Matrix	1.8×10^{-6} M	Ortolani et al. (2019)

Bio-Thiol Sensor

Cysteamine, Cysteine, and glutathione are bio-thiols present in the biological system. For differentiating biothiols in human plasma, the sensor work by visual discrimination mechanism mainly by fluorometric technique. Abbasi-Moayed

et al. (2018) refined a ratio metric fluorescence (RF) sensor array for the selective visual sensing of the biothiol. This sensor was made with N-Acetyl L-Cysteine capped green CdTe quantum dots, Red CdTe QDs-CDs, and Rhodamine B (GQDs-RhB) (RQDs-CDs) on BC nanopaper as radiofrequency sensing elements. Thiol sensor as-prepared is capable of separate biothiols as well as their rapid/accurate identification plasma from humans.

Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit	Reference
BC	Fluorometric	Matrix	8×10^{-6} M	Ortolani et al. (2019)
BC	Fluorometric	Matrix	300 μ M	Abbasi-Moayed et al. (2018)

Bacteria Sensor

Bacteria Sensor gives a quick diagnosis of bacteria that can be used right at the point of care. The 3D porous structure of CNC facilitates dense and uniform adsorption of plasma nanostructures. Tian et al. (2016) used BC as a flexible SERS substrate. BC has a substantially reduced surface roughness than typical filter paper, ensuring high SERS activity uniformity throughout the entire substrate. This resulted in SERS signal enhancement than the plasmonic paper. So, the 3D BC-based SERSs can be used to recognize and detect bacteria such as *Escherichia coli* (*E. coli*).

Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit	Reference
BC	SERS	Substrate	N/A	Abbasi-Moayed et al. (2018)
BNC	BET	Matrix	N/A	Ajdary et al. (2022)

Chemical Sensor

Ion-Selective Sensor

Ions are crucial because they can be used in a variety of disciplines, including clinical toxicology, the food industry, waste treatment, and environmental standards. Ion-selective sensors are often less expensive and easier to use than existing instrumental analyses like AAS, ICP, etc. Ion-selective sensors must be designed with adequate sensitivity, selectivity, stability, and adaptability with aquatic environments in mind. Nanocellulose-based films can improve sensor stability, reusability, and recyclability, as well as the morphology and optical

characteristics. Lee et al. (2007) during the study of fluorescence emission of pyrene observed that the fluorescence emission increased when modified with CNC, as a result of excimers formation. Based on this observation, he designed a sensor that shows high selectivity for Fe^{3+} with particular discrimination between Fe^{2+} and Fe^{3+} . In a novel fluorescence-based sensor, electrostatic adsorption and/or covalent bonding are effective ways to bind fluorescent compounds to nanocellulose. Rajawat et al. (2013) used anodic stripping voltammetry to construct a sensor for determination of cadmium and lead ion in water samples, for which a carbon paste electrode (CPE) was effectively constructed on the CNF. At an accumulated duration of 10 min, the detection limits for lead and cadmium are 33 and 88 g L^{-1} , respectively, which is attributable to CNF's good metal ion adsorption ability due to the presence of functional groups (carboxylate, hydroxyl, and aldehyde groups). Weishaupt et al. (2017) used cyanobacterial C-phycocyanin (CPC) as the sensing biomolecule inserted in TEMPO-oxidized CNF as the matrix to create a sensor to detect copper ions. Here, the amount is based on copper concentration on CPC fluorescence emission. Because of their consistent morphological and optical properties, the CNF-containing CySense films were made efficiently and may be examined using a variety of techniques, including fluorescence spectrometry. Pouzesh et al. (2019) constructed a plasmonic sensor for optical sensing of cyanide (CN) ion by embedding copper nanoparticles (Cu NPs) in nanocellulose films. The nanoparticle size is influenced by the concentration of precursor ($\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$), which in turn determines the maximum absorbance. Chauhan et al. employed pH-sensitive azo-dye-grafted CNC to create an optical pH sensor. The vinyl sulfone reactive moiety of the azo-dye-grafted CNC is employed to take advantage of the intrinsic reactivity of pure CNC and minimize the degradation of CNC morphology brought on by mechanical processes. The pH sensor, printable inks, and pH-sensitive inks are made using CNC-dye conjugate. A pH sensor composed of polyrhodamine-coated CNC that changes color reversibly in response to pH changes was also demonstrated by Tang et al. By employing distinct cross-reactive sensor elements, array-based sensing of target analyte in mammalian olfactory and gustatory systems is capable of producing unique fingerprint patterns for each analyte. As a result, it can recognize and distinguish a variety of target analytes as they produce unique fingerprint patterns. Abbasi-Moayed et al. reported a ratiometric fluorescent sensorarray (RFSA) with BC nano paper as a flexible and transparent substrate. The BC nanopaper was treated to produce hydrophobic walls with laser printing, due to the interaction between heavy metal ions and carbon-dots-rhodamine B(CDs-RhB)nanohybrids it produces color changes. The resulting sensor array can discriminate between metal ions such as Hg^{2+} , Pb^{2+} , Cd^{2+} , Fe^{3+} , and Cu^{2+} . When different heavy metal ions are added to the BC nanopaper-based RFSA, it displays various colors, which can be recognized using RGB analysis for their distinctive, fingerprint-like photoluminescent response patterns.

Sensor type	Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit	Reference
Fe^{3+}	CNC	Fluorometric	Matric/binder	$1 \times 10^{-6} \text{ m}$	Tian et al. (2016)
Cd^{2+} and Pb^{2+}	CNC	Voltammetric	Adsorbent	$88 \mu\text{g L}^{-1} (\text{Cd}^{2+})$ and $33 \mu\text{g L}^{-1} (\text{Pb}^{2+})$	Pouzesh et al. (2019)
CN^-	CNC	Colorimetric	Matrix	$0.015 \mu\text{g mL}^{-1}$	Zhang et al. (2012)

Metal Nanoparticles-Selective Sensor

Sensor type	Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit	References
AgNPs	CNF	Fluorometric	Gelator	$11.1 \times 10^{-6} \text{ m}$	Rajawat et al. (2013)
AuNPs	CNF	Fluorometric	Framework	$1.42 \times 10^{-6} \text{ m}$	Ruiz-Palomero et al. (2016)

Metal nanoparticles are likely to discharge into the environment, which poses serious threats (Ruiz-Palomero et al. 2016) to biological species and human health. As a result, developing effective and efficient ways for determining their identification is critical. Ruiz-Palomero et al. (2016) used carboxylated nanocellulose and $\text{Ru}(\text{bpy})_2(\text{a-bpy})(\text{PF}_6)_2$ to create a highly luminous hydrogel-based sensor for AgNPs, with nanocellulose acting as a gelator. Wang et al. (2018) developed a carbon quantum dots (CQDs) functionalized CNF hydrogel for detecting AuNPs in aqueous solutions, with the hydrogel acting as a 3D framework and the CQDs as the fluorescent donor. The quenching of CQD fluorescence by AuNPs resulted in the sensing capabilities of CQD-functionalized CNF hydrogel toward AuNPs.

Organic Dye Sensor

The water bodies near textile industries are increasingly polluted due to the organic dyes. So, accurate determination of the organic dye is a need of time. Nanocellulose can also be employed as a reducing and stabilizing agent, providing significant stability and allowing plasmonic nanoparticles to monodisperse in solutions without aggregating. Since nanocellulose can be utilized as a framework for loading metal nanoparticles and as substrates for SERS because of its huge surface area and high aspect ratio. Ogundare and van Zyl (2019) developed a malachite green (MG) detecting sensor based on AgNPs/ SiO_2 /CNC nanocomposite, where CNC plays the role of both reducing and stabilizing agent in the synthesis of AgNPs.

The silica was made via a sol-gel technique, and the CNC kept the AgNPs from aggregating. The AgNPs/SiO₂/CNC nanocomposite is proven to be an excellent substrate for fungicide and insecticide SERS.

Sensor type	Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit	Reference
MG	CNC	SERS	Reducing/stabilizing agent	0.9×10^{-9} M	Wang et al. (2018)
MG	BC	UV-visible spectroscopy	Adsorbent	0.4–3.1 μ M	Vilela et al. (2019)

Gas Sensor

The primary sources of pollutants that regularly impair natural environmental conditions include growing population, industrial emissions, automobile exhaust, and open burning of rubbish debris. To avoid environmental degradation, ongoing monitoring of these contaminants is required. There is a variety of equipment available to monitor pollutants and dangerous gases, but they are time-consuming, costly, and rarely employed in real-time situations. For gas sensors, hierarchically porous aerogels are used for gas adsorption and storage which increase the possibility of improving the contact surfaces between analytes and sensing materials. The ability of 3D structures to improve gas sensing capability over their 2D counterparts has been widely shown, which may be ascribed to more reactive sites, the greater specific surface area, and bigger “space” for the storage or movement of electrons/holes and gases.

According to Wang et al. (2008) TiO₂ nanostructured sheets have O₂ sensing capability with sensitivity down to sub ppb levels. The film thickness and operation temperature have an impact on the gas detecting characteristics of the film. When the concentration is more than 1 ppm, the nitric acid becomes the cause of photochemical smog and acid rain which can be harmful for humans. Sadasivuni et al. (2016) used CNC sheets with iron oxide on the surface to create a flexible and reversible resistive NO₂ gas sensor. CNC provides good NO₂ molecule accessibility to the sensor surface, resulting in a highly sensitive sensor. CNC can also facilitate and stimulate the creation of an unbroken linked channel between iron oxide particles, hence increasing the electrical percolation sites and resulting in a stronger response than plain iron oxides and also sensor’s recoverability.

Koga et al. (2019) devised a paper-based molecular sensor based on paper, NO₂ sensor with graphite drawn from pencil as an electrode. Here, the device was constructed on a CNF paper substrate, the ZnO nanowire was tailored via the papermaking process to ensure the attachment of the nanowire to the substrate and accessibility of the analytes. Due to the cost-effectiveness and air stability, graphite is deposited by using a drawing process onto the ZnO nanowires/CNF nanopaper.

When exposed to NO_2 , the electrical resistance of the paper sensors increases, with good sensitivity.

Dai et al. (2017) have developed an ammonia gas sensor based on copper (II)-doped CNC cholesteric films. Due to the CNC's optical activity and nematic chiral signature, ammonia gas interacts with CNC and the chelated Cu^{2+} and the nematic layers of CNC and, a redshift in reflective wavelength and an effective colorimetric change occur. Narwade et al. (2019) investigated the efficacy of hydroxyapatite (HAp) nanoparticles blended CNF films with various topologies for NH_3 sensing. The films with 5% HAp have a detection limit of 5 ppm, a sensitivity of upto 57.5%, and a response/recovery time of 210/30 s, which is much superior to the HAp and CNF counterparts. The explanation for this is the increase in conductivity caused by the creation of H bonds between the OH groups of cellulose and the NH_3 molecules.

Zhang et al. (2017) worked on creating an I fuel-cell for alcohol sensing utilizing a CNF/graphene oxide (GO) membrane. The sensor reacts to ethanol vapor with a detection limit of 25 ppm at varying doses. In addition to being responsible for in-plane ion-transport channels of the CNF/GO membrane and through-plane and, the grafted sulfonic acid groups on the surface of GO and CNF nanosheets with 3-mercaptopropyl trimethoxysilane as a precursor are also in charge of proton conductivity. Because of their linear chain molecular structure with repeating glucose units and numerous exposed surface hydroxyl groups, CNF may be easily changed to transmit protons or hydroxide ions.

Wang et al. (2019) with PANI and carboxylated ginger-derived CNF fabricated a supramolecular aerogel (SA) with a porosity of 96.90% and conductivity of 0.372 mScm^{-1} . With carboxylated ginger-derived CNF and PANI. He created a conductive supramolecular aerogel (SA) with a porosity of 96.90% and a conductivity of 0.372 mScm^{-1} . Because its resistance changes with different gases and the response curves have distinct forms, the SA was also used as a gas sensor to detect ethanol, formaldehyde, chloroform, and toluene. The higher electrical resistance is produced by the conformation change and expansion of PANI chains which is caused by the assault of chloroform and ethanol molecules on the N-H sites. Toluene, on the other hand, produces electrons when it enters PANI, reducing the number of positive charge carriers and neutralizing positrons.

Sensor Type	Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit	Reference
NO_2	CNF	Fluorometric	Matrix	N/A	Ogundare and van Zyl (2019)
Benzene, toluene and chlorobenzene	BC	Resistance	Matrix	N/A	Wu et al. (2019)
Chloroform, ethanol, toluene, and formaldehyde	CNF	Resistance	Matrix	N/A	Wang et al. (2019)

Toxic Compound Sensor

For detecting biological and chemical analytes, SERS has long been considered a label-free analytical tool. Nanocellulose-based planar materials (films, membranes, and sheets) make up 2D nano cellulosic materials. Microporous structure, flexibility, lightweight, thermal/mechanical robustness, chemical stability, and portability, are the characteristics of nano cellulosic material. They are also predicted to be utilized in the development of portable and disposable sensors. Since nanocellulose has a high aspect ratio and specific surface area it is a good substrate for SERS. (Chen et al. 2019) fabricated in situ non-invasive pesticide detectors in fruits and vegetables with the help of SERS. In this sensor, the jelly-like AgNPs were decorated on the nanocellulose surface, the SERS method shows a 0.1 ppm for thiram and 1 ppm for thiabendazole. A silver nanocellulose composite-based substrate for carbendazium detector was developed by Huang et al. (2019) A homogenous silver nanocellulose composite was developed by reducing silver and hydroxyl group on the surface of the nanocellulose (Morales-Narváez et al. 2015). Fontenot et al. (2017) proposed a colorimetric sensor for the detection of hazardous substances such as thiourea and cyanide by using gold nanoparticles (AuNPs) embedded in BC nanopaper (i.e., thiourea and cyanide). With the addition of thiourea, the AuNPs-BC changes from red to dark red due to the aggregation of AuNPs generated by thiourea. When cyanide is added the nanoparticle shrinks and the color changes from red to light pink.

Ruiz-Palomero et al. (2017) developed a fluorescence sensing hydrogel by embedding S, N-codoped graphene quantum dots (S, N-GQDs) on nanocellulose, in which the S, N-GQDs act as a luminophore and sensitizer, while the nanocellulose as the matrix for hydrogel. When GQDs are added to nanocellulose hydrogel as opposed to GQDs solutions, there is a considerable increase in fluorescence intensity because of stability of GQDs without aggregating in the hydrogel and the strong dispersity. The resulting GQDs/nanocellulose hydrogel was further explored for selectively sensing of 2,4,5-trichlorophenol due to a significant rise in photoluminescence intensity of GQDs while interacting with TCP (TCP). The photoluminescence intensity of GQDs is greatly increased as a result of these interactions. Furthermore, the hydrogel formation enhances the contacts between TCP and GQDs, resulting in high fluorescence emission. The photoluminescence intensity of GQDs is greatly increased as a result of these interactions. Furthermore, the hydrogel formation enhances the contacts between TCP and GQDs, resulting in high fluorescence emission.

Sensor category	Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit	Reference
2,4,5-trichlorophenol	CNC	Fluorometric	Matrix	0.07 $\mu\text{g mL}^{-1}$	Liu et al. (2019)
Thiram and Thiabendazole	CNF	SERS	Substrate	Thiram (0.1 ppm) and	Chen et al. (2019)

(continued)

Sensor category	Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit	Reference
				thiabendazole (1 ppm)	
Carbendazim	CNF	SERS	Substrate	0.01×10^{-6} M	Chen et al. (2019)

Drug Sensor

For studying the pharmacokinetics of drugs and risks with the usage of drugs, drug detection, and monitoring are needed. The examination of human biological materials such as urine, blood, perspiration, saliva, and the exhaled breath is commonly used for drug detection and monitoring. Because of their great sensitivity, cheap cost, and quick analysis timeframes, electrochemical sensors are ideal for evaluating pharmaceuticals in pharmaceutical preparations and human bodily fluids. The flexible 3D-nano cellulosic includes hydrogels, aerogels, and sponges that are porous structures so they can combine with nanoparticles and nanostructures in sensing. The porous structure and various routes for analytes diffusion and sorbent capacity improve sensor performance. Hydrogels made from nanocellulose offer a range of chemical, mechanical, and biological properties as well as a high water retention capacity. For example, combining transparent nanocellulose hydrogel with the optical characteristics of a fluorophore might result in a high-sensitivity optical sensor. Lightweight biocompatible materials having structural features that are compatible with highly exudative wounds, such as nanocellulose-based aerogels/sponges, are appropriate for biosensor design. The structural stability of nanocellulose-based aerogels is often poor, resulting in the collapse or closure of interconnecting micropores. Furthermore, because of the poor connection between fluorescent and the aerogel skeleton molecules, fluorescent components aggregate and quench, resulting in lower fluorescence performance.

Morales-Narvaez et al. (2015) developed a coulometric sensor for the detection of methimazole by using the AgNPs-BC system. Due to the good optical transparency, high mechanical strength, flexibility, and high surface area of BC nano paper, it is used as an optical sensing platform. In the presence of methimazole, the color of the AgNPs changes from yellow to dark yellow because the thiol group in methimazole act as a linker promoting the aggregation of Ag NPs. For iron-chelating drug molecules, Faham et al. (2019) synthesized a calorimetric sensor based on a BC nananopaer. The curcumin-embedded BC nanopaper shows a complexation of Fe (III) and curcumin in the presence of Fe(III) which shows a decrease in the intensity of the color. With the addition of an iron-chelating drug, the intensity of the color can be recovered. Liu et al. (2019) used BC as a template to make p-type NiO nanoparticles for sensing volatile organic compounds such as benzene, toluene, and chlorobenzene. To produce NiO nanoparticles, BC and nickel acetate tetrahydrate

were mixed to form gels, which were then freeze-dried to produce nickel-adsorbed BC xerogels, which were then calcinated in air. Wu et al. (2019) created fluorescent aerogels containing CNF and CDs that can be employed for nitric oxide (NOx) detection since NOx may quickly quench the fluorescence. The cause of fluorescence quenching is the suppression of radiative recombination of electrons and interactions between NOx and CDs. The porous nature of aerogel aids in the adsorption and trapping of gas molecules.

The glassy carbon electrodes were modified using CNF-CNPs (carbon nanoparticles) nanocomposite films to create an electrochemical sensor (GCE). Electrodes were used to detect trace quantities of metoclopramide in clinical and pharmaceutical preparation. The modified electrodes work effectively for measuring minute amounts of metoclopramide (MCP) in therapeutic and pharmaceutical formulations. In comparison to bare GCE, these produced modified electrode that have a 49-fold better anodic response to MCP. A nanocellulose/functionalized-multi-walled carbon nanotube (f-MWCNTs) composite film based biosensor has been prepared for the sensing of nonsteroidal antiinflammatory medication, diclofenac sodium (Shalauddin et al. 2019). Nanocellulose has a lot of hydroxyl groups that can be used as binding sites for various analytes. Furthermore, the synergy of nanocellulose and f-MWCNTs increases the sensitivity and selectivity of electrochemical detection.

Sensor type	Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit	Reference
Metoclopramide	CNF	Amperometry	Substrate	6×10^{-9} M	Huang et al. (2019)
Diclofenac sodium	CNC	Amperometry	Substrate	12×10^{-9} M	Ruiz-Palomero et al. (2017)
Iron-chelating drugs	BC	Colorimetric	Substrate	8.2×10^{-9} M	Shalauddin et al. (2019)

Physical Sensor

Proximity Sensor

A proximity sensor works without any physical contact with the analyte object. This can be integrated into any intelligent technology like guards, robots, wearable electronics, biomedicine or during tissue engineering. The sensor works on the principle of infrared emission, electromagnetic induction, and resistance measurement. Sadasivuni et al. (2015) presented a proximity sensor from ecofriendly and transparent CNC/rGO which can be used in both non-touch and touch screens which has the ability to detect finger approaches. Isophorone diisocyanate (IPDI), acts as a functional agent on GO and it also act as a coupling agent for CNC and GO. The CNC/GO composite was then reduced using anhydrous hydrazine. CNC influences

the semiconductor characteristics, which has an impact on sensing performance. As the finger approaches the sensor, a little electric charge transfer disturbs the fringe electric field, resulting in the detecting property. The sensor's quick reaction and great sensitivity may be ascribed to the CNC/GO composite's chemical modification and reduction, as well as its outstanding nanostructure. Remarkably, the sensors operate better under the combined influence of CNC and rGO than under the management of rGO alone.

Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit	Reference
CNC	Resistance	Substrate/spacer	6 mm	Morales-Narváez et al. (2015)

Temperature Sensor

Temperature mapping and monitoring are necessary for many heterogeneous environments such as medical fields. Flexible substrates such as nanocellulose can be used for the temperature sensor. Jung et al. (2017) created a thermoelectric sensor based on CNF, the thermocouple was made by inkjet printing thermoelectric materials (such as CNTs, AgNPs, and PEDOT-PSS) onto CNF films. The CNF substrate absorbs the conductive materials as a result it acts as a suitable substrate for inkjet printing. Vuorinen et al. created another temperature sensor by using an electrohydrodynamic inkjet (E-jet) printer and printing AgNPs on BC, which has a 10 times greater resolution than standard inkjet printers. This skin-conformable temperature sensor has a sensitivity of greater than 0.06% resistance change per degree Celsius.

Nanocellulose category	Sensing technique	Role of nanocellulose	Detection limit	Reference
CNF	Resistance	Substrate	N/A	Faham et al. (2019)
CNC and CNF	Fluorometric	Dispersant/substrate	243 K	Sadasivuni et al. (2015)

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Packaging Applications of Biodegradable Nanocellulose Composites

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Sanjiv Sonkaria, Kwang-Hyun Ryu, Varsha Khare, and
Hyun-Joong Kim

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Abstract

Ensuring the safe disposal of waste materials to prevent irreversible environmental damage is a major priority concern of the current century. The alarming rate of consumption of plastic-based packaging materials has steadily increased and plastic accumulation now seemingly contributes to the global carbon footprint substantially adding to greenhouse gas emissions. While it is difficult to reduce the global dependency on plastics as a cheap and easily polymerizable material source, nature provides a sustainable alternative to process waste into eco-friendly breakdown products that are recycled back as nutrients by microorganisms and are not harmful by-products. Packaging in the form of naturally occurring biodegradable matter is becoming an increasingly attractive alternative to replace common nonbiodegradable plastic. Biopolymers assembled in nature are by their intrinsic nature biodegradable but retain some ability to exhibit plastic-like properties. Hence in this review, the knowledge gap that currently

S. Sonkaria · V. Khare

Soft Foundry Institute, Seoul National University, School of Engineering, Seoul, South Korea
e-mail: ssonkaria64@snu.ac.kr; khare@snu.ac.kr

K.-H. Ryu · H.-J. Kim (✉)

Smart Center for Adhesion Research & Development (Smart CARD), Laboratory of Adhesion & Bio-Composites, Program in Environmental Materials Science, College of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea
e-mail: hjokim@snu.ac.kr

prevents the use of readily available natural biopolymers namely nanocellulose that have the potential to be engineered into biodegradable plasticized products is highlighted. This review aims to focus on discussion points that are underrepresented with regard to aspects on nanocellulose. Here, bond fragility of nanocellulose is one concern limiting its use as a viable biodegradable bioplastic. Material failure has its origins in chemical bond breakage and while much progress has been directed toward experimental investigations of nanocomposites formed from nanocellulose, the connection between an intrinsic understanding of chemical bonds with structural-functional nature of nanocellulose is lacking. Here, we explore these in more detail in our efforts to build a greater appreciation to broader areas covered elsewhere.

Keywords

Biodegradability · Nanocellulose · Biopolymers · Recyclability · Renewability · Microplastics

Introduction

Importance of Biodegradable Packaging

Cheap packaging has been synonymous with the birth of plastics since the turn of the twentieth century and has since symbolized the face of durability, strength, and tough polymer chemistry. The oversupply of the “make, consume, and dispose” business model is symbolic of a huge disposal waste problem for an industry worth \$450 billion dollars. Now decades into the new millennium, the future is firmly embedded by a plastic-dependent existence with overwhelming consequences to the environment and human and animal life by diminishing the eco-benefits of a plastic-free world. Plastic production and its disposal are major climate change issues and the world's dependence on plastics promises to be a significant growth driver by industries. While plastic has favored an economic advantage to the consumers and industries alike particularly in terms of packaging of products, recyclability has not featured heavily in the “plastic” business model largely due to the cost burden. Insight into the sense of scale of plastic consumption is appreciable from an expected drop of 100,000 tonnes if its utilization was met by a 50% reduction. In reality, less than 10% (Rahimi and Garcíá 2017) of plastics are recycled with little prospect of depolymerizing the most toxic plastics that contain liquids and food products. Figure 1 shows that the plastic industry is predominately driven by the packaging industry and this dominance is set to increase and plastic utilization (Fig. 1; green bubble) is not expected to slow down. The most popular plastics are related polyethylene low density (PE LD), polyethylene high density (PE HD), polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET).

More than nine billion metric tons of plastic have been manufactured since the 1950s of which 40% represents single-use plastics. Such industry-led challenges

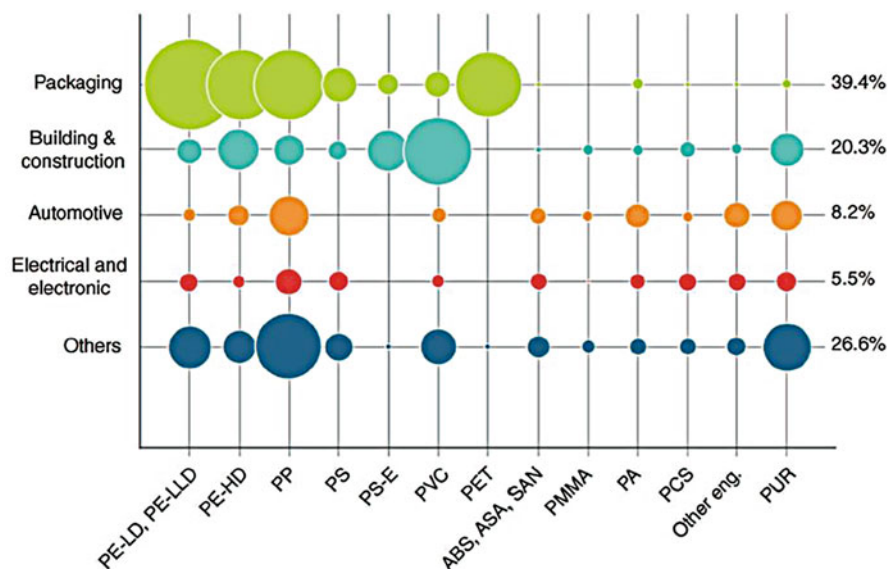


Fig. 1 The dominance of the packaging sector among other industries as a leading consumer of synthetic plastics. (Reproduced with permission from Lackner 2015)

pose a prime environmental concern of growing catastrophic proportions originating from technological gaps in the disposal of nondegradable plastic packaging. The accumulation of plastics in landfills impacts both land-based and marine animals as water-dissolved plastic waste is carried by rain water deeper into soils groundwater that connect streams, rivers, and oceans. This permits the systematic poisoning of all with types of ecosystems it encounters into a collective patch of plastic debris that accumulate into the Great Pacific Garbage Patch (GPGP), a vortex of marine debris which was surveyed to be the size equivalent to 1.6 million km² in 2018 (Lebreton et al. 2018). Plastics of varying size including millimeter-sized plastics find their way within marine animals and into the human food chain. Microplastics have been identified in table salt, tap water through water contamination, and synthetics like nylon and polyester can release thousands of microparticles into our laundry. It was also found that plastic feeding water bottles and injectors released as much as 393 particles/mL just from repeated opening and closing cycles (Song et al. 2021). Figure 1 shows that common cutting processes like scissoring, tearing, and cutting of packaging results in the release of microplastics demonstrated by the mass change (Sobhani et al. 2020). Further, microfragments originating from other everyday plastics like tearing of bag of chocolates, sealing tape, and bottle caps were visualized under SEM and the morphologies were correlated to FTIR and QCM analysis of freed microparticles by the tear (Fig. 2). More recently, examination of human feces for the existence of ingested microplastics in the guts of humans from a group of a small group of disease-free study subjects aged 33–65 years led to the identification of ten types of microplastics. Polypropylene and polyethylene terephthalate were

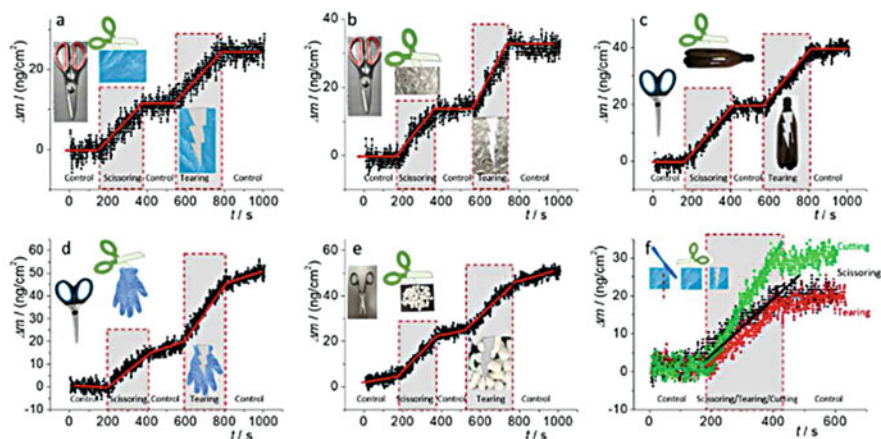


Fig. 2 Microplastics generated from scissoring, tearing, and cutting of plastic packaging. Increase in mass associated with microplastics was measured by quartz crystal microbalance. (Reproduced with permission from (Sobhani et al. 2020). This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0))

correlated to nine of the study subjects as the most commonly occurring type and eight samples revealed microplastics ranging from 50–500 μm in size (Schwabl et al. 2019). Microplastics have been shown to exert their effects which are cell dependent (Caldwell et al. 2021) and new methodologies have evolved to follow the biological effects of nanoplastics as small as 50 nm. Particle imaging by stimulated emission depletion (STED) (Fig. 3 RHS b, d, f and h) shows the shapes adopted by common in the form of nanoplastics which could not be resolved by confocal microscopy (Fig. 3 LHS a, c, e, and g) (Nguyen and Tufenkji 2021). With specifics to a model study using the commonly used packaging material polypropylene (PP), the biological fate of fluorescently tagged PP was followed in zebra fish embryos and was shown to be localized in the gut (Lee et al. 2022). This brings into awareness the existence of submicroscopic particles where the short-term and long biological effects on potential disease development and progression are completely unknown. The persistent nature of plastic packaging is a complex problem of global proportions. Implementing safeguarding strategies against accumulating plastics is energy demanding and current approaches may not be applicable in handling highly toxic plastics like polyvinylchloride (PVC).

The petroleum and fossil fuel origin of the most durable plastics and their future sustainability is a global concern. While plastics are indispensable for the important purpose they serve in packaging of a diverse range of products, the physical, chemical, and mechanical properties do not allow their recyclability at the end of their life cycle. The recyclability and renewability of synthetics are inefficiently low resulting only in partial degradation, the energy demands for breaking down the polymer structures is very high. For example, thermolytic processes that drive polymer breakdown require a high-energy input and control over product

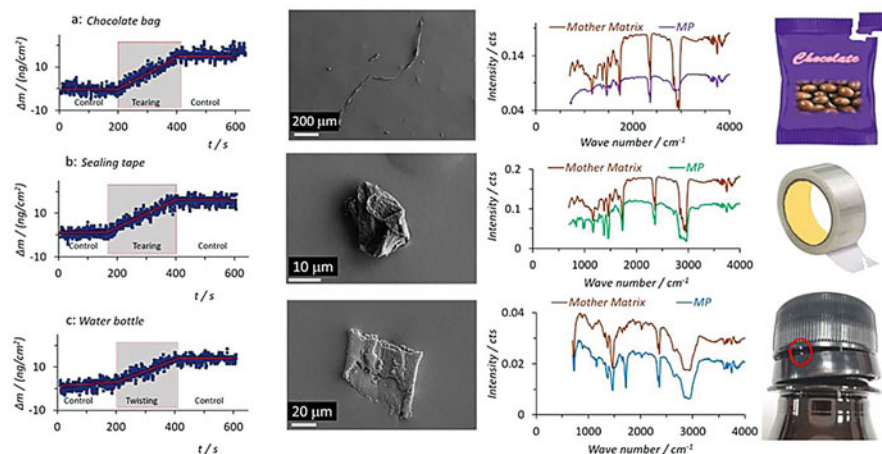


Fig. 3 Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) correlate to microfragments of plastics measured by quartz crystal microbalance (QCM) from tearing of plastic packaging of (a) chocolates, (b) tape, and (c) bottle cap. (Reproduced with permission from (Sobhani et al. 2020). This work is licensed under a Creative Commons Attribution 4.0 International [CC BY 4.0])

degradation is variable depending on the nature of the polymer due to strong bonding. However, thermoset polymers used in the rubber industry were recently engineered using polydicyclopentadiene as model for inserting cleavable cross-linked bonds to render the polymer degradable without affecting its mechanical properties (Shieh et al. 2020) which is a promising alternative to recycling of polymers (Hamad et al. 2013) which require areas of technological advancement to address the chemically diverse and different toxicities often associated with nonbiodegradable plastics and their degraded products. Work toward the design of building blocks that may function at the unit level of monomers and cross-linkers as reactive networks in polymers (Winne et al. 2019) could potentially in the future be used to take advantage of mechanical and physicochemical properties that facilitate depolymerization (Rahimi and García 2017). Currently synthetic polymers are not naturally cleavable in nature but instead were designed to durable and tough.

There is great demand for sustainable packaging which is both cost-effective and eco-friendly. At the leading edge of this endeavor is biodegradable packaging. For any plastic to be biodegradable, the material composition must be chemically and physically accessible to living entities such as microorganisms which can actively recognize chemical groups and release enzymes that specifically digest bonds into monomers and oligomers. The process is sustainable by virtue of the nature of the degradation products since they are a source of nutritional value and energy for the diverse ecosystem of living microspecies that inhabit our planet. Many of the synthetic plastics can potentially serve this purpose as a rich source of hydrocarbons as a feedstock for organisms that hugely outnumber the small number of core synthetic materials in circulation today. Such materials of high molecular weight

include polyethylene (PE), polypropylene (PP), polyvinyl chloride, (PVC), polystyrene (PS), polyethylene tetrathalate (PET), and polyurethane (PUR). While findings suggest that some bacteria and fungi can adapt their biological machineries to degrade synthetic polymers, biodegradation rates are poorly inefficient against the rising accumulation of plastics. However, factors such as structure, molecular weight, surface morphology, mechanical strength, and varying degrees of crystallinity/amorphicity effect degradation which reflect the overall bioavailability of the polymer. Natural or formulated semisynthetic biopolymers are currently of value. Biodegradable and semibiodegradable polymers are attractive targets for the replacement of plastics but lack durability and toughness of plastics. Examples include polycaprolactone (PCL), poly(lactic acid (PLA), polyethylene succinate (PES), polyhydroxyl butyrate (PHB), butylenes succinate (PBS), polyhydroxy alkanoate (PHA), and poly(butylenesuccinate-co-adipate) (PBSA), among others. Another distinguishing factor between synthetics and biodegradable materials is the occurrence of functional group sets that likely influence the broader reason for differences in their bioavailability.

With a greater focus on biodegradable materials, naturally produced environmentally compatible materials are in demand as packaging materials. Rising population and consumer demand is proposing a new technological vision for packaging being actively responsive to its environment particularly to microbes and such intelligent packaging may evolve into sophisticated sensing technologies equipped with indicators interacting with the surrounding atmosphere. Therefore, a strong mandate for “next-generation” materials are alternatives that are easily adaptable to different environments with the ability to be efficiently degraded or converted back into some form of usable packaging. Material availability, renewability, processability and ease of modification for improvement, disposability through efficient recycling, and eco-friendliness and biocompatibility are characteristics that are highly attributable to a class of biodegradable materials termed biopolymers found in nature. Their alignment with diverse microbial communities has evolved mechanisms to break down biodegradable polymers into digestible constituents and satisfy many of the abovementioned requirements.

“Sustainability” and “renewability” are umbrella terms synonymous with natural and biodegradable materials. While renewable materials impose a cost factor from natural sources such as sugar and ethanol, the abundance of natural biopolymers could potentially bring advantages to reduce cost barriers. However, the scale to which biopolymers will eventually need to be manufactured to meet the current and future consumer demands is challenging. The difference among biodegradable materials is the rate at which they degrade. The ideal targets are weak molecular structures that undergo disintegration. Nanocellulose is one such biopolymer. With consideration to renewability, nanocellulose as a bioplastic has numerous physical and chemical exploitative properties and can provide opportunities to introduce new and improved functionalities. However, much of the enthusiasm has been hindered by the cost of mass processing but expectations continue to be high (Lindström and Aulin 2014).

This chapter examines the broader potential of nanocelluloses as a feasible option for replacing petroleum-based plastic packaging and discusses these advancements in reaching the longer-term goals of bringing nanocellulosic materials closer to marketable quality. As plastic has been seen to revolutionize polymer chemistry, by the same token the future packaging materials will be looking to bring newer innovations and packaging will have a multifunctional purpose attached to it. To advance this, a thorough grounding of the fundamental properties of nanocellulose is essential to allow its modification to become a true innovative material for decades to come.

Nanocellulose

Potential Use of Nanocellulose in Packaging

Nanocellulose is probably one of the most interesting plant biodegradable biopolymers that exist. It is abundantly available from wood biomass, plants, and other inexpensive vegetation sources. The market value of nanocellulosic a raw material is expected to reach USD 963 million by 2026 (Markets and markets, 2022) and marks the increasing trajectory of global interest toward biobased renewables. An important requirement for material renewability is its degradable value for energy or food for organisms that rely on it. Because of the increasing demand for packaging as the global population expands, from the perspective of renewability it is also important to consider materials that can be degraded at rates higher than disposal rates to maintain a feasible cycle of replenished packaging material. This aspect directs to material performance and may well be an engineering challenge for biopolymer-synthetic material optimization. Material competency may have a direct or indirect relationship among parameters of surface modifiability, processability, and by-product degradation with overall efficiency and utility in packaging applications. For example, surface modification to provide rigidity to the nanocellulose framework for utility as a plastic may have implications at the biosynthetic interface in biosynthetic polymer blends. Structural and chemical synergy of nanocellulose as part of a blend can result in stiffness, strength, ductility, and toughness (Mautner et al. 2018) and depolymerization is a trade-off between mechanically strong plastics and biosynthetics resisting biodriven degradation. Figure 4 shows nanocellulose composed of unit size structural elements which form covalent bonds and extend into nanodimensional fibers. The large molecular weight can hinder the control of the biopolymer which is largely influenced by viscosity, hydrophobicity, and aggregation. The utility from a commercial point of view is very limited particularly if biopolymers suffer from fragility and brittleness and poor mechanical properties. Enhancement in the surface area facilitated by nanoscale dimensions provides heightened exposure and opens considerable opportunities for interaction with the surroundings. The intrinsic properties of the biopolymer strands can thereby be tailored to desired outcomes with a host of different materials and their blends.

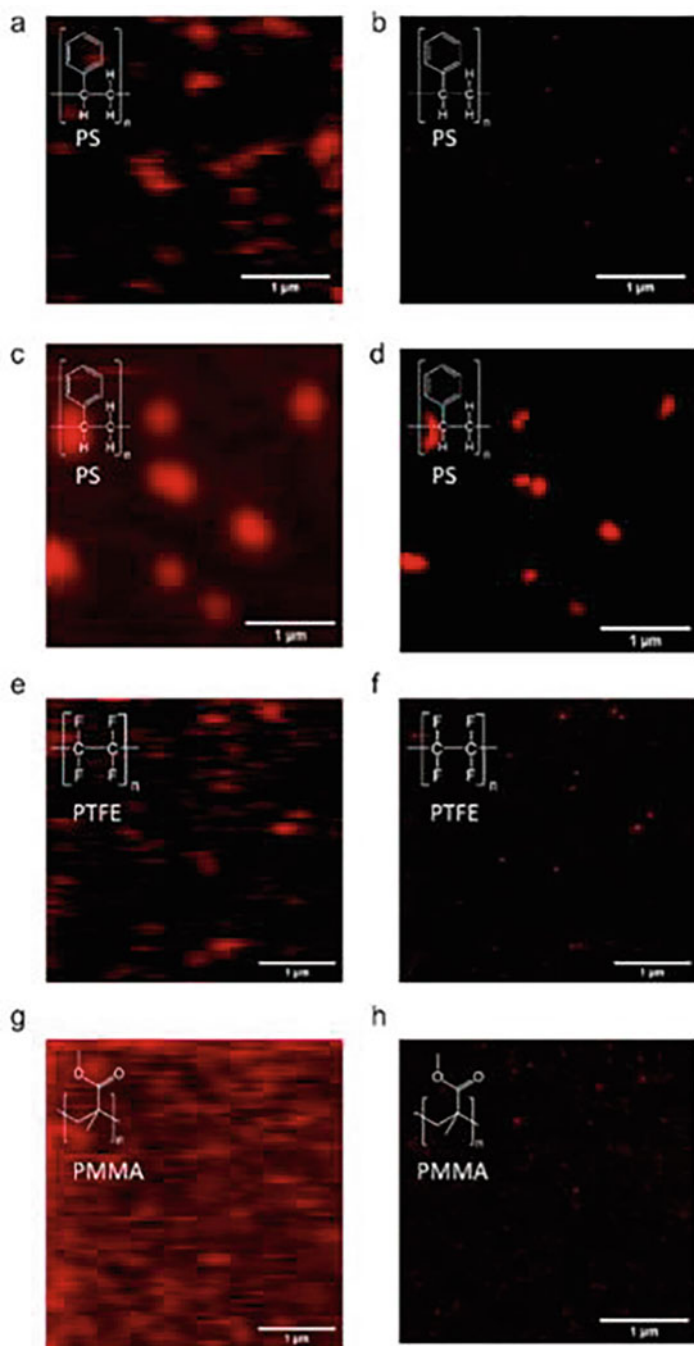


Fig. 4 Fluorescently labeled of differently categorized nanoplastics using confocal (LHS)-stimulated emission depletion (STED) microscopy. (Reproduced with permission from (Nguyen and Tufenkji 2021). This work is licensed under a Creative Commons Attribution 4.0 International [CC BY 4.0])

A Brief Look at Degradation from a Biopolymer Bonding Perspective

Since nanocellulose is a biopolymer, its origins like all polymers such as polypeptides (proteins) and polynucleotides (RNA and DNA) are embedded in biological chemistry as it exists as a polysaccharide (chained sugars) linked by β -1,4-linked d-glucose units. This suggests that such polymers are intrinsically adapted to fold and minimise degradation unlike linear native conformations that more readily degrade in aqueous environments (Runnels et al. 2018). Interestingly Runners et al., describe the resistance to degradation of biopolymers as a possible mechanism operated through “kinetic trapping” of nonequilibrium states favored by folded assemblies. From the perspective of employing nanocellulose for biodegradable packaging, it would therefore be strategically essential to ensure folding states of biopolymers of this type are still accessible to degradation by microbial communities by preventing misfolding under aqueous conditions and that properly formed “transition states” are recognized by enzymes for degradation to occur through accelerated hydrolysis. It should therefore be a primary goal to develop new technologies that reduce the persistence of nondegradable states during processability of the bioplastic. Hence, the linkage stereochemistry and fiber elongation signified by the β - versus α -anomer states of biopolymerization is essential to its biocongruence bearing structural complementarity to enzyme active sites also engaged in reversing polymerization. Figure 5 describes the self-complimentary behavior that extends to all biopolymers (but illustrated by cellulose). Here, the network of hydrogen bond donors and acceptors ascribe to an important role that does not only result in precise alignment of the multilayer structures but also the chemical space generated within polysaccharides through linkage chemistry. Occupation of the chemical space by plasticizers and compatibilizers often have profound effects on both physical and chemical properties affecting the entire structure in the blend. Biopolymer properties

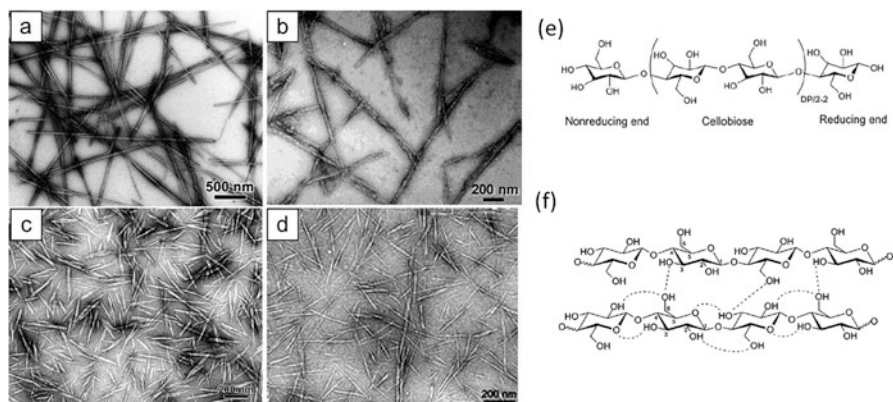


Fig. 5 A TEM representation of images of nanocellulose nanocrystals obtained from (a) tunicate, (b) bacterial, (c) ramie, and (d) sisal. (e) Molecular assembly of cellulose chains. (f) Hydrogen bonding in nanocellulose with water molecules. (a–e) Reproduced with permission from (Qiu and Hu 2013) (f) Reproduced with permission from (Pinkert et al. 2009)

of cellulose at the submicroscale has been the focus of attention in numerous works and more relevance to the macroscale properties in correlation to chain dynamics of monomers has been lacking. Local flexibility, for example, is fundamental to identifying how unit scale behavior drives collective behavior at the macroscale where ultimately their utilization in applications will rely on a globalized structure. Structure-property relationships, however, are useful for predicting outcomes and can guide polymer engineers to more precisely tune materials in mapping three dimensions with biopolymer function. Scanning tunneling microscopy and electro-spray ion-beam deposition have been particularly useful in this regard in correlating levels of hierarchical structure in terms of sequence and chain confirmation of the biopolymer chain of cellulose with local flexibility. In a recent study using cellulose as a model (Anggara et al. 2021), changes to the primary structure through substitutions that affect intermolecular hydrogen bonding provided a series of archetypes to investigate chain flexibility, molecular conformation, and local secondary structure. The changes to the unmodified cellohexaose represented by [AAAAAA] as the native structure constituted substitutions defined by [ABAABA], [ACAACA], [ADAADA], and [AFAAFA] where A is Glc, B is Glc methylated at OH(3), C is Glc methylated at OH(3) and OH(6), D is Glc carboxymethylated at OH(3), and F is Glc deoxyfluorinated at C(3). Figure 6 demonstrates that the sugar chains for the

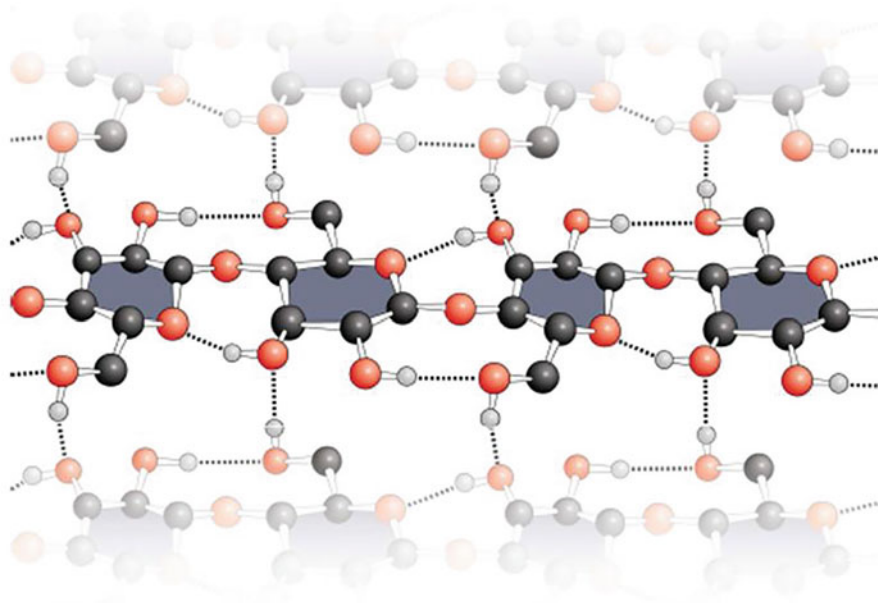


Fig. 6 A schematic illustrating the stability of cellulose by the hydrogen bond donor-acceptor network of complementary units which polymerize along the β (1–4) directional folding path. (Reproduced with permission from (Runnels et al. 2018). This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0))

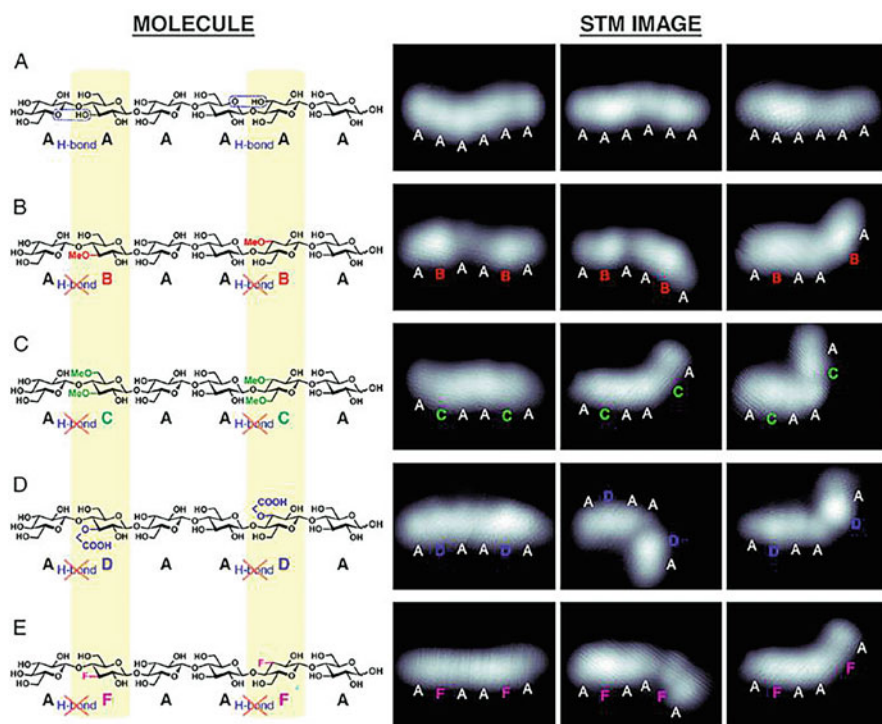


Fig. 7 Native and modified cellohexaose imaged by scanning tunneling microscopy (STM) showing the effect of substitutions on chain flexibility conforming to structures A, B, C, D, E, and F. Taken from (Anggara et al. 2021). This work is licensed under a Creative Commons Attribution 4.0 International License

native structure is linear and the modified chains are more flexible as they can adopt both straight and bent geometries. The conformation of the chain is defined by structural association between adjacent sugars with respect to their relative geometries. Figure 7 shows twisted and untwisted chains and its importance to the degree of chain flexibility. The corresponding angle measurements (Fig. 7, B, C and D) differentiate between H and V states of the Glc sugar separated by height of the ring either in a parallel or perpendicular orientation to the surface with respective H and V. The twisted linkages were determined to exhibit greater chain flexibility compared to untwisted chains. Twisting directly diminishes the interaction between adjacent Glc units and increases flexibility. The freedom to bend results from the exclusion of intermolecular hydrogen bonds and stresses the importance of extrinsic chemical factors that have the potential to alter the folding patterns of biopolymers affecting their ability to resist or enhance aggregation and interaction with key molecules like water. The study also revealed that mechanical parameters like stiffness is likely a consequence of modifications to intramolecular interactions and less influenced by surface-to-surface interactions.

Functional Group Similarity and Dissimilarity Between Synthetics and Biodegradable Polymers

The highly abundant hydroxyl moiety [OH-C] pronounced within the group chemistry of nanocelluloses positions this material as one of the most natural and easily biologically accessible functional group structures on the planet. It is not surprising that this single structural feature that is an integral part of the unique assembly of nanocelluloses appears to be commonly shared among natural product functional group repertoire displayed in Fig. 10. The overall polymer reactivity is connected to surface hydroxyl groups and their relative positions largely determine the biopolymer characteristics.

Since functional group in polymers renders them degradable or nondegradable and is the chemical epicenter of the polymer, it may be meaningful to correlate the occurrence of functional groups characteristic of its nature. It may be important to understand the chemical properties of semisynthetic polymer as a result of a blend with unknown behavioral properties. Dominant functional groups become important to the identity of a complex blend and so much can be inferred about the overall chemical nature of the material and behavior. Identifying patterns of behavior through molecular structure analysis using analytical approaches can also be supported by cheminformatics (Ertl 2017) and machine learning (Nalla et al. 2018) routes. Cheminformatics has been insightful in highlighting differences in the distribution function groups in natural systems in comparison to synthetic ones. In a recent computational study designed to identify differences in functional group occurrence, alcohol hydroxy group (61.1%), alkenes (39.9%), ethers (35.2%), esters (28.7%), and phenolic hydroxy group (28.4%) feature most commonly (given by the frequency percentage in the parenthesis) and the corresponding structures are shown in Fig. 8 (Ertl and Schuhmann 2019). It is not surprising that oxygen is the dominant atom that forms a part of the hydrocarbon structural backbone and is well aligned to

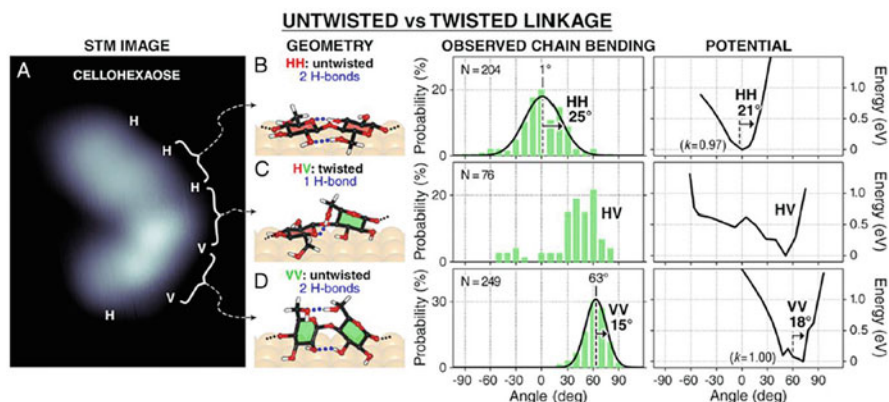


Fig. 8 (A) Cellohexaose showing two types of AA linkages, untwisted (HH and VV) and twisted (HV and VH). Corresponding bending angles and the computed potential curve are shown in B for HH, C for HV, and D for VV. Taken from (Anggara et al. 2021). This work is licensed under a Creative Commons Attribution 4.0 International License

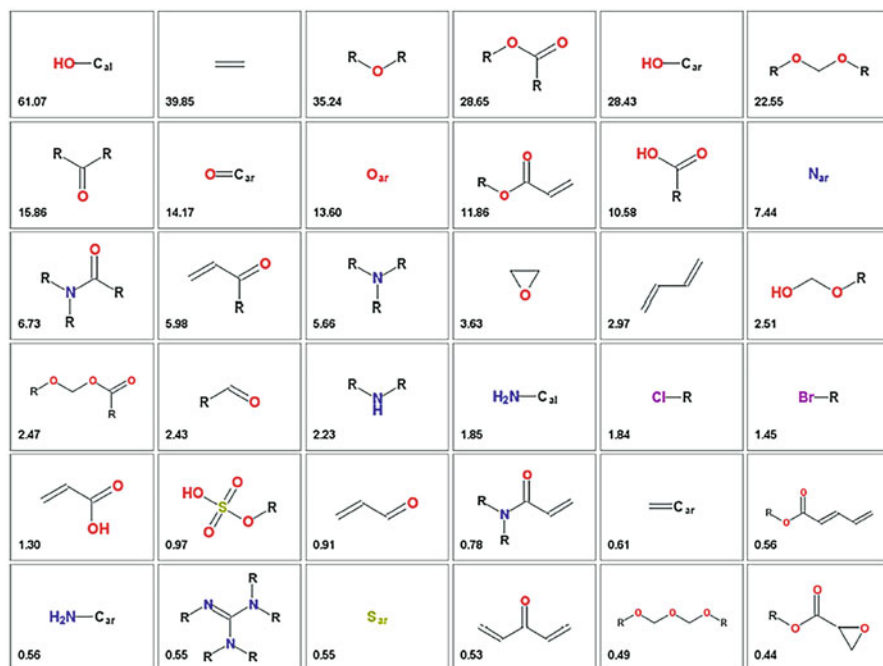


Fig. 9 The most prevalent functional groups found in nature. (Reprinted with permission from [Ertl and Schuhmann 2019]. Copyright [2019] American Chemical Society)

enzyme active sites of biodegrading enzymes. This signifies that preoxidation of synthetic plastics is a necessary prerequisite to their biological degradation and the inclusion of oxygen likely reduces the energetic barrier relative to nitrogen bonds. Of greater relevance to assess the extent of the dissimilarity between natural and synthetic functional plastics is presented by Fig. 9 which highlights the distribution of the most frequently identified chemical functional groups. The plot reflects the diverse distribution of biodegradable functional materials that are of value in the microorganism food chain as natural substrate polymers while others are allied to the synthetic type. It can be reasoned that microbes show preference for biodegradable polymers which fall in the region of functional groups circled in Fig. 5. Polyethylene and polypropylene, for example, show a strong carbon-hydrogen backbone and are not readily degradable due to major differences in the functional groups that define characteristics and behavior. Polyethylene terephthalate, for example, also show different competency to biodegradability that reflects the variation in the functional groups and enzyme accessibility to the structure is made possible through exposure to UV light by photooxidation or hydrolysis. For synthetic polymers, functional group accessibility is followed by depolymerization. Table 1 summarizes the mode of trigger applied to different PEs and the functional groups isolated were identified. In the microbial environment, increased carbonyl index was observed during the biodegradation of LDPE over a

Table 1 Summary of functional group types after triggered degradation

Degradation trigger	Functional group	Category of polyethylene
UV	Aldehydes, Ester	HDPE, LDPE, and LLDPE
	Ketones	
Xenon	Carbonyl	LLDPE
	Acid	
Electron beam and gamma	Ester, ketone	HDPE
Photooxidation	Esters and lactones	PE with vinyl and t-vinylene groups
Thermo-oxidation	Ketones and acids	
Accelerated environmental conditions	Aldehydes, ketones	LDPE and LLDPE
Environment	Esters	Environmentally beached PE pellets
	Ketones	
Natural	Ketones and acids	HDPE
	Carbonyl	

Adapted from (Fotopoulou and Karapanagioti 2017)

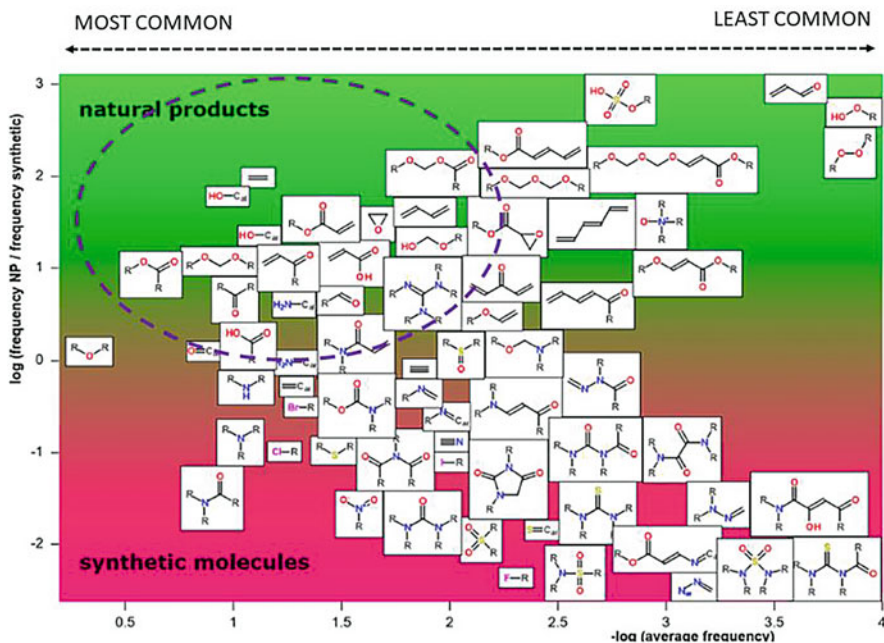


Fig. 10 Distribution of functional groups in relation to frequency of occurrence and their propensity toward natural (green) or synthetic (red). (Reprinted with permission from (Ertl and Schuhmann 2019). Copyright (2019) American Chemical Society)

period of years with weight loss. Under accelerated growth, carbonyl products are also formed resulting from increased surface cracks and increased fragility during the timescale of the exposure period (Fotopoulou and Karapanagioti 2017) (Fig. 10).

Formulating Bioplastics: Considering Pre/Post-Extraction Phase of Nanocellulose

The purpose of bioplastics is one that drives an environmental goal but also attains to an economic one which while satisfying the greener concepts of sustainability, renewability, and biodegradability of eco-bioplastics can replace synthetic plastics without incurring a cost burden against the cheaper alternative to conventional plastics. International standards for biodegradation for environmentally degradable plastics and biobased products sets performance standards which must be met to categorize bioplastics as environmentally acceptable (ASTM D6954 2013). While CO₂ and evolution methane (CH₄) are the important measurement criteria from biodegradation, oxygen consumption has also been suggested to be important for aerobic biodegradation (De Wilde 2012). Currently, bioplastics are in the infancy of development despite the considerable research-based publications which point to considerable potential to formularize a truly biodegradable bioplastic of wide applicability. In this direction, nanocellulose is a popular target and example of the scope of blended products as semisynthetics. The prospects of using a naturally occurring biopolymer as a base material for sustainability while introducing strategically informed modifications to improve performance plastic replacements in an economic way are already underway. Currently, semisynthetics resembling bioplastics for packaging (Ibrahim et al. 2021) have their origins in biopolymers which are subjected to modification by chemical processes via abiotic routes (Ganie et al. 2021). Figure 11 emphasizes some of the performance outcomes the future envisages from technologically engineered bioplastics.

Nanoscale cellulose exists as cellulosic nanofibrils (CNFs) and cellulosic nanocrystals (CNCs) and these forms typify nanocellulose and are extractable by a combination of physical (e.g., mechanical homogenization) and chemical treatments (e.g., acid hydrolysis) (Phanthong et al. 2018). Acid hydrolysis can affect the hydrogen bond network and has been inferred to cleave on intramolecular hydrogen bonds at the macromolecular level on the O(3)H...O(5) bonds (Li et al. 2021). Morphological analysis also reveals bond breaking and bond reordering was

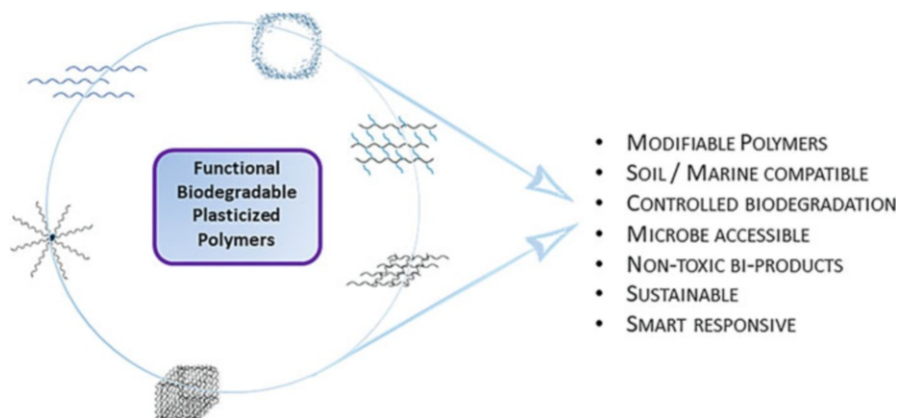


Fig. 11 A schematic summary relating different biodegradable polymers

Table 2 Relationship between nanocellulose type and dimensional size

Natural fibers/Plants	Type of nanocellulose	Dimensions of extracted nanocellulose	
		Length	Diameter
Eucalyptus pulp	CNC	150 nm	10–50
Pinecone	CNF	–	5–20
Soybean straw	CNF, CNC	>1 μ m	10
Hyacinth fiber (<i>Eichhornia crassipes</i>)	CNC	147.4 nm	15.6
Pineapple leaf fiber	NC	<(88–1100) nm	68
Rice hull	CNF	–	<100
Banana rachis, kapok, pineapple leaf, and coir	CNF	–	10–25
Oil palm trunk	CNF	170–800 nm	4–10
Bamboo	CNC	–	50–100
Waste paper	CNC	100–300 nm	3–10
Banana peels	CNF	455 nm	10.9
Palm residue	Coir NC	–	108
	Fronds NC	–	90
Coir fiber	CNF	–	37.8
Jute fiber	CNF	–	50
Beer industrial residues	NC	–	73–145
Coconut residues	CNF	–	70–120
Silkworm silk fiber	CNF	–	0.1–0.4
Flax	CNC	57 nm	6
Cotton	CNC	68 nm	8

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accompanied by reduction in size. This change relates to the opening of the glycosidic bond which provides opportunities for reordering. Ultrastructural differences among nanocelluloses can indeed affect susceptibility by acids (Palme et al. 2016). A high variability in structural dimensions and shape and size can result from the source of the biopolymer and pretreatment methods used for extraction which affect yield, crystallinity, and can potentially tailor desired morphological, surface chemistry, and numerous physicochemical outcomes governed by methodology employed. Table 2 shows that the dimensional size of extracted nanocelluloses is largely source dependent (Khalid et al. 2021). Hence, chemical and mechanical processes can introduce profound changes to the structural properties of the biopolymer through pre- and posttreatment regimes which at the molecular level interfere with the inter- and intramolecular bonding (Fig. 12). It is necessary to understand the implications and consequences of these alterations before using nanocelluloses from different sources due to the broad variability in properties. For example, the crystal packing and the degree of crystallinity or amorphicity can comprise the integrity of the nanocellulose structure if penetrating water molecules can enter the chemical spacing between adjacent units and compete for bonds and thus reducing mechanical properties.

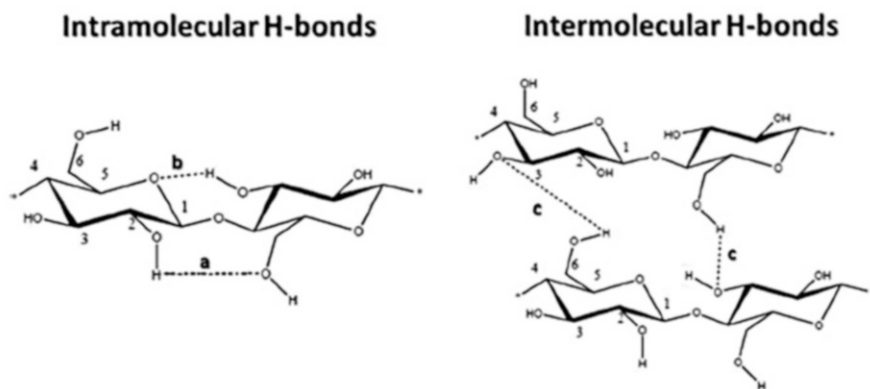
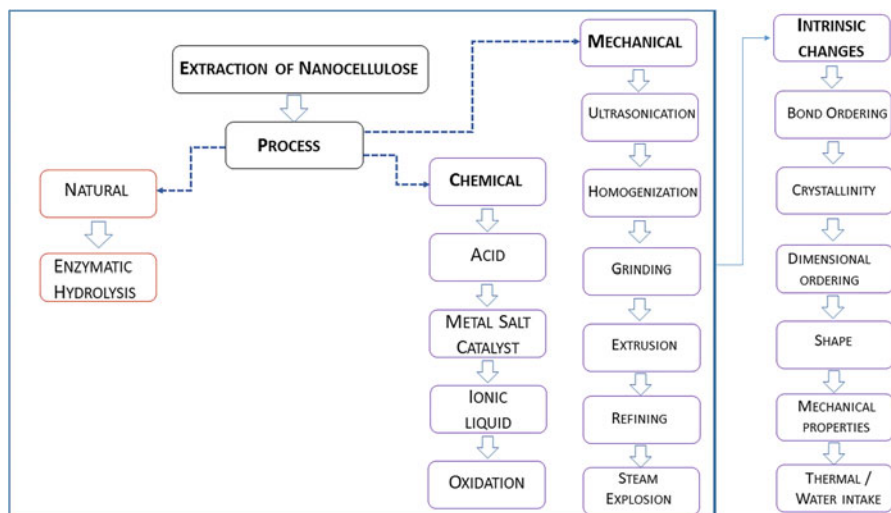


Fig. 12 Structural arrangement of intra- and intermolecular bonding in cellulose. Reproduced from the thesis entitled “Interfacial Forces in Nanocellulose-Based Composite Materials” (Olszewska 2013)



Scheme 1 Extraction processes for nanocellulose and the effect of intrinsic changes induced by external processes. (Adapted with permission from [Khalid et al. 2021]. This work is licensed under a Creative Commons Attribution 4.0 International [CC BY 4.0].)

However, in well-dispersed reinforced polymer nanocomposites, cohesive interaction between nanocellulose crystals offer less resistance to degradation by solvents and thus highlights the understanding of cohesive particle–particle interactions (Reid et al. 2016) and meets the design for bioplastic packaging. Blending with other materials may affect the nature and magnitude of forces such as van der Waals, hydrophobic, hydrodynamic, electrostatic (between layers), repulsive, and steric forces. The current challenge is to find a structural and functional balance of material combinations that are mutually synergistic with each other (Scheme 1).

Considerations of Nanocellulose from Nature's Perspective: Future Implications for the "Brittleness" Problem for Biodegradable Bioplastics

Much of the research for nanocellulosic materials has been devoted to engineering strategies for modification of the biopolymer surface with synthetic materials as blends. Such studies for packaging applications (Abdul Khalil et al. 2016) have centered on reinforcement for enhancing compatibility (Wang et al. 2021), dispersion (Chu et al. 2020), interfacial adhesion (Wolf et al. 2013), processability (Souza et al. 2020), stability (Santmartí and Lee 2018), moisture resistance (Cherpinski et al. 2018), and tensile strength to improve mechanical properties (Talebi et al. 2022). Some studies have escalated to provide active and intelligent functionality and smarter nanocellulose materials (Heise et al. 2021) including sensing (Fuertes et al. 2016) toward more innovative packaging. However, there has been little discussion focusing on using nanocellulose for biomimetic composites. Much of the emphasis has been to find reinforcements for nanocellulose as a biosynthetic composite to solve issues of fragility particularly in its interaction with water.

In nature, nanocellulose relies on its hierarchical existence to achieve many of the above properties that would readily endorse its use as a packaging material. This suggests that clues to mechanical optimization lie within the hierarchical makeup of the biopolymer. A very small body of knowledge has emerged that is pointing to improve our knowledge of structure and function which nature has inherently designed to be more adaptive than rigid. This could be taken to support the notion that natural materials may succumb to stress imposed upon them by the environment, but such stresses seldom destroy the material in its entirety or irreversibly. In contrast, mechanical and chemical processes can impose considerable stresses through deformation or fracture which primarily leads to bond loss.

Only under extreme deformation, irreversible disintegration can occur. Hierarchical cooperation from the nano- to macroscale exists and requires further appreciation. It is plausible to say that an understanding of material hierarchy is not understood such that the mechanical deformation at one scale due to hidden interconnections can affect the properties at other scales in the same material. Under bearable loads, biopolymers are capable of adjusting material responses that allow multimechanisms to operate which defines the robustness of a material. In this respect, studies that relate chemical bonding networks of natural structures and mechanical properties offer greater potential for new technological outcomes.

Bioinspired Biopolymer Synergy

Reduced toughness is a potential technological flaw in applying nanocelluloses for long-term applications in packaging for durability. This weakness is characteristically attributed to the lack of deformation – the mechanisms of which are still largely unaccounted for at the molecular level. Needless to say, attention has been given to improving the mechanics of the biopolymer in the form of composites with numerous synthetic molecules that bear many of the desirable mechanical properties biopolymer fall short on. The delicate balance between toughness and biodegradability is viewed as a critical setback of structure-function relationships. Lessons in sustainability of biopolymers rest in understanding how mechanisms unfold under normal

environmental conditions through the use of structure-sensitive tools which could better inform researchers of the behavioral aspects of nanocelluloses.

Surface cross-linking of nanocellulose takes advantage of the abundant hydroxyl group moieties inherent to its structure. The prevalence of hydroxyl groups in nature demonstrated by the functional propensity and frequency plot of Fig. 10 predicts that the probable deviation from its assigned role in nature might drastically reduce nature's ability to degrade the biosynthetic blend. This is particularly true if nitrogen-containing synthetics are blended within the nanocellulose structure as judged by the functional group content of plot 10. Consideration between physical- and chemical-based cross-linking of hydroxyl groups also give rise to concerns over “stronger” versus “weaker” arguments and implications of bond strength in resulting blends. In recent times, it has been rightly recognized that the surface interactions of nanocelluloses are far from trivial. This is certainly true of water vapor adsorption at the amorphous/crystalline interface that exists as a 1 nm layer (Niinivaara et al. 2015) and the availability of hydroxyl groups can affect its ability to swell to control permeability which may affect its use technologically (Capron et al. 2017).

Poor moisture resistance of nanocellulose is perceived to be major barrier to technologically advancing in the area of packaging and related areas (Cherpinski et al. 2018) and the sensitivity to humidity is professed to an unresolved issue. The persistence of humidity in the environment has a tendency to drive deformation and induce a loss of mechanical stability. Often, the material complexity of nanocellulose blended materials circumvent a better understanding of the accurate nature of the interaction of water vapor in the surroundings with the molecular intricacies residing in the biopolymer structure itself. In hindsight, the mediated effects of moisture are elusive in the midst of reported observations that support the role of water as a plasticizer (Mittal et al. 2018). The computational study revealed that while the presence of intercalated water molecules could be unfavorable universally for the global performance, a considerable change in shear modulus fits in with its bio-inherent ability of hierarchical fiber networks to respond to changes in the vapor content of the environment in the same way as a fabricated sensor might. In Fig. 13, simulation trajectories predict the molecular importance of water molecules (Chami Khazraji and Robert 2013) by forming associations termed “capillary bridges” at the nanocellulose interface and serve to increase the fracture energy by

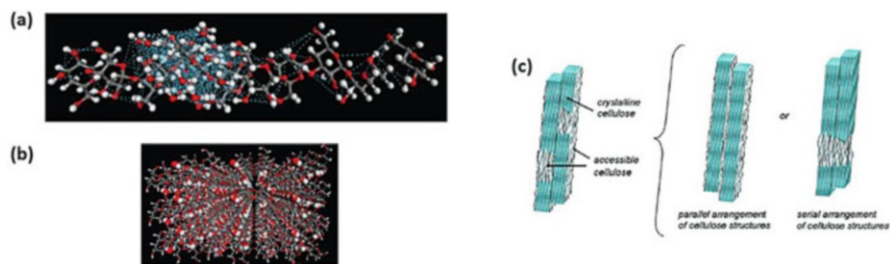


Fig. 13 (a, b) Simulation of water molecules at the cellulose nanocrystal interface showing preferential contact between water molecules. (a, b) Taken from (Chami Khazraji and Robert 2013) (c) (Salmén and Bergström 2009).

acting to increase the interface prior to failure. Further, strain force has greater toleration under moist conditions compared to dry ones and a parallel-type arrangement of water molecules could be conducive to load-bearing operations (Salmén and Bergström 2009). This suggests water has a bigger role to play.

Greater relevance might come from deciphering the hydrogen bond network with interconnections to water molecules with the aim of predicting more precisely the role of water molecules under differing atomical arrangements. This scenario should reflect the competition for CNC binding sites that likely occur between CNC and water molecules. To this effect, with increased accessibility of water molecules, the study considers that under conditions of humidity, CNC–CNC bonds are intercepted by water molecules and rearrange to a water intercalated arrangement, CNC–water–CNC. A combination of multiscale simulations and experiments were applied to investigate differences between hydrogen (HBs), CNC–CNC (nanocellulose), and CNC–water–CNC bonding arrangements under variably defined conditions for humidity at the interfaces. The study is insightful in that it provides a relative percentage of humidity which is correlated to monitor changes in the mechanical properties mapped for all three bonding arrangements. The main findings of the study signify the formation of a denser hydrogen-bonded network for CNC–water–CNC and MD simulations and surprisingly strain-stress profiles comply with strain reinforcement applying to both tensile stress and the strain at break. Relative humidity (RH) falling in the range $30\% \leq \text{RH} \leq 50\%$ reveals the relative condition for the strain hardening phase which is lost to a weaker modulus and interfacial strength when $\text{RH} \geq 60\%$ resulting from a higher accumulation of water (Hou et al. 2021). Hence, depending on the conditions, the water-intercalated nanocellulose interface can switch between a mechanically strong and weak interface by adjusting to the environmental conditions in sensory fashion. Figure 14 shows the tensile stress curves and number of bonds as a function of water, and Fig. 15 shows tensile stress curves, tensile stress as a function of relative humidity profiles, and SEM images of fracture morphologies under variable conditions of humidity. Hence, self-modulation of mechanical properties in response to conditions of stress and strain hierarchy in nanocellulose has a multifunctional role that reaches far beyond the confines of structure.

Stress transfer between two different interacting components can serve a useful purpose for enhancing mechanical strength. Almost universally, strength increase is achieved at the cost of strain and toughness which is true for most nanocomposites. However, this mutually exclusive relationship is contradicted by increases in strength, elongation, and toughness. With brittleness as a primary problem of nanocelluloses, bioinspired strategies have proven to generate remarkable results for high-performance nanocellulose-based materials. In one study, a combination of two biopolymers comprising bacterial cellulose nanofibers (BC) and alginate were combined into a bioinspired hierarchical helical shape. The hydroxyl moieties of BC were readily accessible to the anionic polysaccharide carboxylate groups of alginates via a single capillary wet extrusion process in the presence of an aqueous CaCl_2 bath under a shear force and combined together under a wet twisted motion. The simultaneous accompaniment of all the three mechanical parameters of strength,

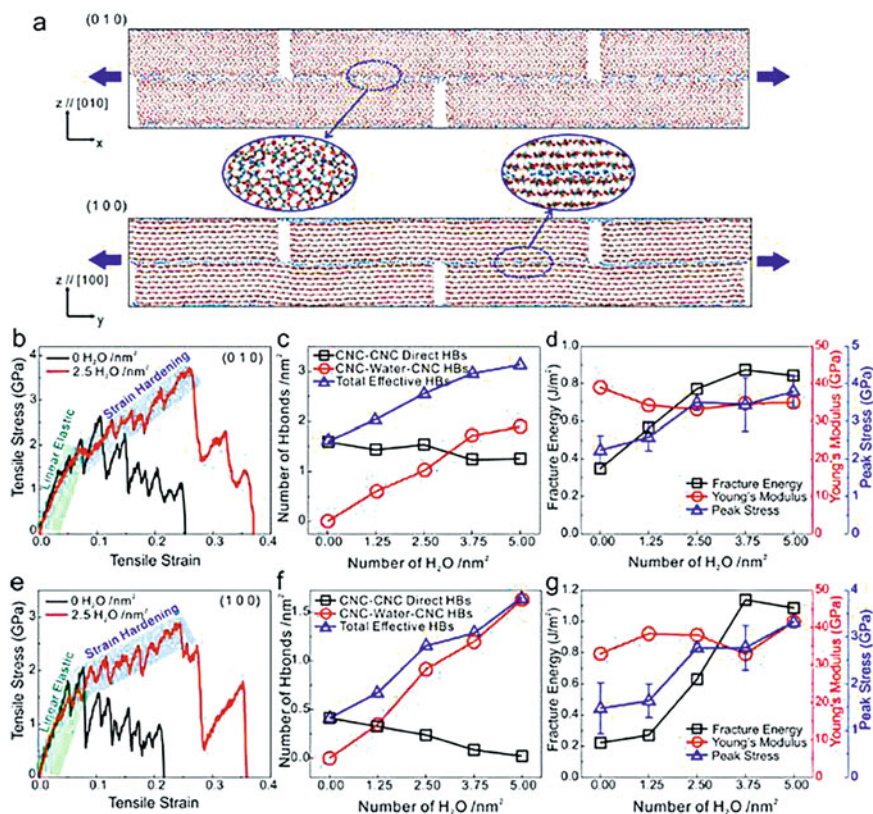


Fig. 14 (a) Humidity-mediated interface mechanical models, (b–d) tensile stress curves, number density of hydrogen bonds (e–g) profiles using the nanocellulose as a model. Blue/Green areas denote elastic stage and hardening stages during tensile process. (Reprinted with permission from (Hou et al. 2021). Copyright (2021) American Chemical Society)

elongation, and toughness were also achieved with increase of 50 and 100% under variable conditions of relative humidity resulting from the twisted synergy of fibers. In contrast, untwisted nanocomposites in their dry states with considerable loss of water experienced inferior mechanical properties compared to the wet gel filaments (Gao et al. 2020). Figure 16 summarizes the fabrication of twisted morphologies of BC-Alg accompanied (a) by TEM and AFM images with height profiling (b–d) and the extrusion of hierarchical structures. Figure 17 summarizes the mechanical properties of BC-Alg morphologies in terms of their stress-strain, tensile strength, and toughness.

Concluding Comments

There is a slow but steady rise toward the development of biodegradable materials while researchers and technologists aim to re-formularize the way we create, utilize, and dispose of plastics. Hence, investment growth in new technologies and

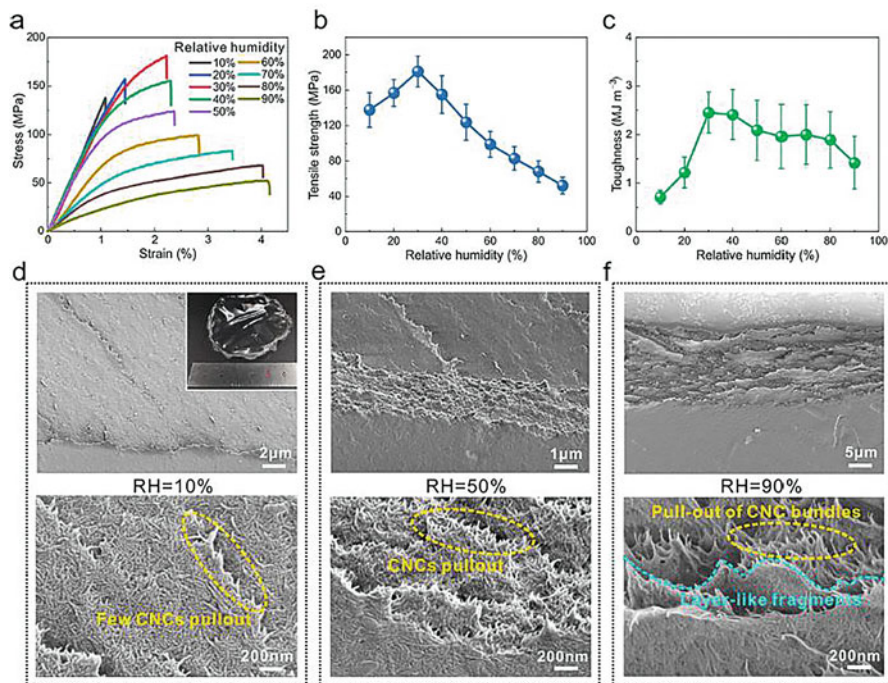


Fig. 15 (a) Tensile stress curve of CNC with 10–90% RH range, (b) Tensile strength measured as a function of RH, (c) toughness as a function of RH, and (d–f) SEM images showing morphology of fractures experimentally obtained under 10%, 59%, and 90% RH, and (d) prepared CNC film. (Reproduced with permission from (Hou et al. 2021). Copyright (2021) American Chemical Society.)

innovative tools is elemental to material renewability and sustainability to visualize a new industry *in* bioplastics. However, till the present day, the situation still remains largely unchanged in implementing safeguarding strategies against accumulating plastics which are energy demanding and may not be applicable in handling highly toxic plastics like polyvinylchloride (PVC). Thus, rethinking the approach to plastic packaging has become a conceptual challenge from design to recyclability of by-products to reduce the carbon print. To strive for a carbon-free world as it relates to nondegradable packaging is unrealistic but the passage back to carbon neutrality necessarily means a bigger commitment to synchronize materials with nature. The conceptuality of this idea is being developed by adapting nonbiodegradable materials or their composites to resemble structurally and behaviorally natural polymers.

Nanocelluloses lie at intersection of bio- and synthetic polymer engineering. As the number of biosynthetic nanocellulose composites reportedly increase year by year, there is small yet concerted effort to better understand structure-function correlations that reside within nanocelluloses. A greater understanding of structure

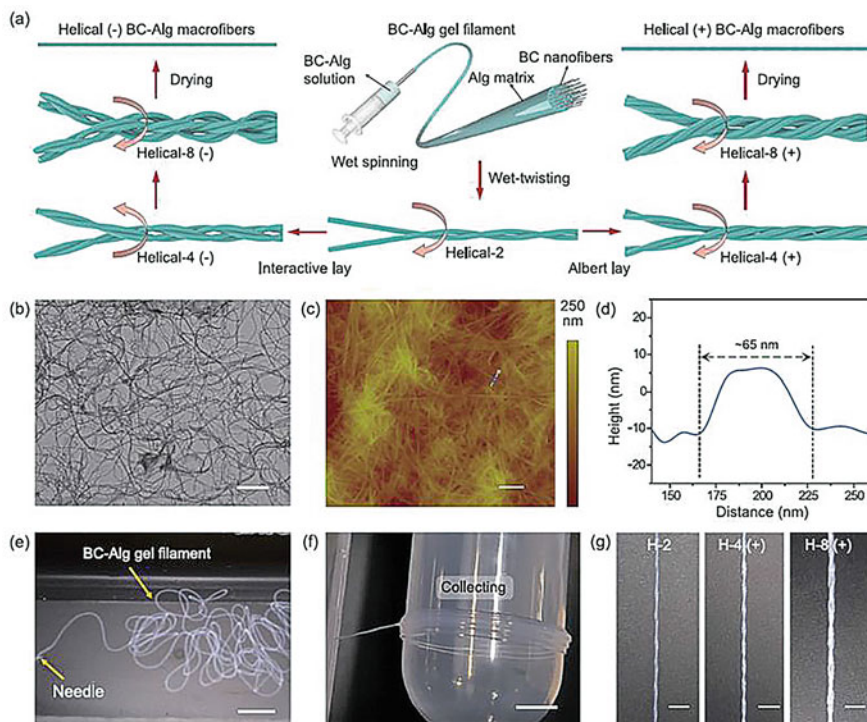


Fig. 16 (a) Fabrication of twisted bacterial cellulose alginate. (b) Transmission electron microscope images of BC nanofibers. (c, d) Atomic force microscope imaging and height profile of BC nanofibers. (e) BC-alginate gel image after its extrusion through the capillary in the CaCl₂ bath. (f) Image of a wind roller used for the continuous BC-Alg filament. (g) Twisted BC-Alg bundle following wet twisting at different hierarchical levels. (Reproduced with permission from (Gao et al. 2020). This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0))

in terms of bond connectivity across all hierarchical scales is key to elucidate performance-related mechanisms as a potential biodegradable material. An important area gaining attention relates to environmentally responsive structural changes that correlate to weak and strong mechanical performance. Switchable states that reflect mechanical performance of nanocelluloses is complex and requires an in-depth study to ascertain the intrinsic capabilities as a bioplastic. One emerging but insightful area has focused on the interfacial properties and behavior with water. A more extensive appreciation of the chemical and structural synergy with its environment and with other biopolymers could provide more effective solutions in challenging area of biodegradable plastics. To this end, the merger of natural resources and technology is imperative. Natural biodegradables offer considerable opportunity to leverage renewable technologies and related areas to improve plastic recyclability and cost reduction.

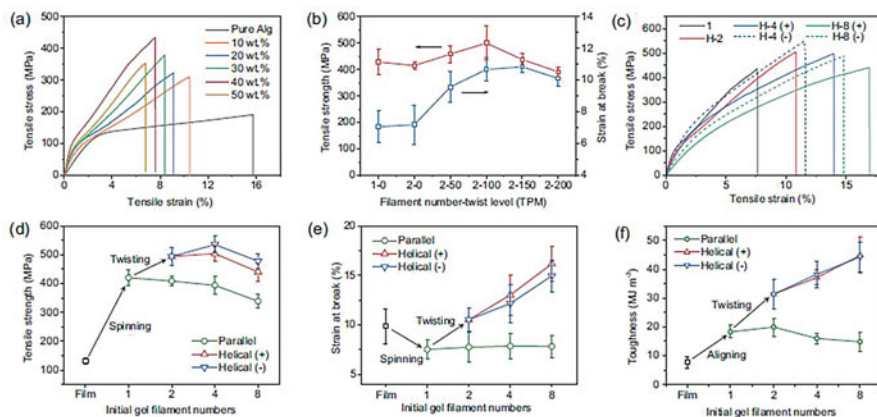


Fig. 17 (a) Stress-strain curves of dried single BC-Alg filaments of different compositions. (b) Tensile strength profiled against twist level of helical –two fibers. (c) Stress-strain curves exemplifying macrofibers of BC-Al hierarchical structures; (d–f) d showing the tensile strength at strain break shown in (e) and toughness in (f) as functions of hierarchical levels. (Reproduced with permission from (Gao et al. 2020). This work is licensed under a Creative Commons Attribution 4.0 International (CC BY 4.0))

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Nanocellulose-Based (Bio)composites for Optoelectronic Applications

37

Roshny Roy, Meenu Eldhose, Cincy George, and Alex Joseph

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Abstract

Electronic devices frequently include flexible circuit boards, and soon the substrate of picture displays will be composed of flexible materials as well. Due to their innate flexibility and optical properties, plastics are potential choices; however, they also have significant thermal expansion. To prevent damage during the thermal cycles required in the production of the display, the substrate's expansion needs to be compatible with that of the active layers that have been placed on it. Reinforcing plastics with nanofibers is one method of lowering the thermal expansion of those materials without significantly reducing transparency. In nature, the primary source of cellulose is plants and animals which is the major source of nanofibers. Here are some of the researches to produce optically transparent composites based on natural nanofibers for use in flexible displays are presented and discussed.

R. Roy · M. Eldhose · C. George · A. Joseph (✉)
Department of Chemistry, Newman College, Thodupuzha, India
e-mail: alex.chiramattel@gmail.com; alex.joseph@newmancollege.ac.in

Keywords

Nanocellulose · Biocomposites · Optical properties · Flexible films · Optical applications

Introduction

Cellulose is the abundant and most commonly used polymer on the earth synthesized by cell-free systems, microorganisms, and plants. It is chemically composed of repeating units of β -D-glucose monomers linked through β -(1,4)-glycoside linkage. A hemiacetal group found at the end of each cellulose chain enables the asymmetry in the cellulose chain's orientation. Nanocellulose can be synthesized in crystalline and amorphous form and can be methodized through a physical, chemical, and/or enzymatic reaction. Natural cellulose has a fibrous shape with occasional crystalline and amorphous portions. It is mostly extracted from cellulose-containing material, with wood being the most common source. Cellulose extracted from the wood source usually has a unique hierarchical and hollow structure. The production of these fibers and their use in composite materials have gained more attention in recent years due to the numerous advantages of nanocellulose, including their high melting temperature, 200 times the amount of surface area, high crystallinity, biodegradability, finer web-like network, and stiffness with low weight (Kim et al. 2015; Eichhorn et al. 2010).

Nanocellulose has gained a lot of attention in recent times due to its interesting properties and potential application in bio-generated and bio-renewable material (Klemm et al. 2011). The nanocellulose often called cellulose nanostructure is classified into three categories namely: (1) cellulose nanocrystals (CNC) also known as nanocrystalline cellulose, cellulose (nano)whiskers, or rod-like cellulose nanocrystals; (2) cellulose nanofibrils (CNF), which include nano fibrillated cellulose (NFC), micro fibrillated cellulose (MFC), or cellulose nanofibers; and (3) bacterial nanocellulose (BNC) also referred to as microbial cellulose.

Nanocellulose due to its distinctive property such as three-dimensional web-shaped reticulate fibrous structure, biocompatibility, good mechanical strength, high crystallinity, hydrophilicity, biodegradability, optical transparency, poly-functionality, high specific surface area, and the ability to be molded into three-dimensional structures. The three-dimensional hierarchical structures that makeup nanocellulose fibers at various scales, the combination of cellulose's physical and chemical properties like hydrophilicity and the ability to be functionalized and chemically modified by a wide range of reactions, as well as the key benefits of nanoparticles such as aspect ratio, high specific surface area, improved mechanical properties, optical transparency and low thermal expansion coefficient all contribute to the development of nanocellulose fibers (Yano et al. 2005; Nakagaito et al. 2010; Nogi and Yano 2008; Okahisa et al. 2011).

However, despite its many advantages, nanocellulose has drawbacks that limit its use, such as incompatibility with hydrophobic polymers, low-temperature stability,

and moisture absorption (Guimard et al. 2007). The current difficulty in using nanocellulose for high-value materials is to add functions through blending, covalent modification, cross-linking, polymerization, or a combination of these approaches. The presence of a significant number of hydroxyl groups inside the nanofiber's structure may act as a planned basis for surface alteration using various substituents, making it extremely hydrophilic and adaptable to a variety of chemical and physical modifications. Nanocellulose has thus been widely regarded as a suitable nanostructure for creating new high-value nanomaterials due to its great biocompatibility, mechanical strength, renewability, and low cost.

The use of nanocellulose in conjunction with carbon-based materials like graphene, carbon nanotubes, and carbon dots has been proposed as a viable strategy for the future of sustainable electronics (Lee n.d.). This new class of hybrid materials with a synergetic effect in a variety of applications, ranging from sensing to biosensing, catalysis, photonics, medical, wearer treatment, optics, and optic energy has also emerged. Some of the less popular nano-carbon materials used in CN-based hybrids are carbon black, activated carbon, carbon quantum dots, and carbon nanofibers (Oksman et al. 2006; Yoon et al. 2006).

A great candidate material to replace hard glass has been considered to be nanocellulose. Nanocelluloses can be used to perform a variety of optical tasks. The topic that has received the most attention is probably that of CNC's cholesteric liquid crystallinity, which produces tunable iridescence, including structural colors, and enables the transfer of such qualities into other materials via a template. Due to the chiral twisting of endogenous cellulose components, some berries can exhibit structural colors (<http://www.oled-display.net/oled-revenues-forecast>). In order to understand tactoid formation or in the case of microfluidic droplets, the formation of chiral nematic phases of CNC was also investigated in spherical confinement in emulsions. These investigations demonstrated the angle-independent photonic characteristics and radial symmetric orientation of the CNC layers. On the other hand, a significant area of nanocellulose-related research has been obtaining transparency in films (Forrest 2004).

A UV visible spectrometer can be used to evaluate the light transmittance in the wavelength range of 200–1000 nm in order to determine the optical characteristics of nanocellulose films. If the fibrils are small enough and packed closely enough, CNF films may be optically transparent. This will prevent light scattering by minimizing the interstices between the fibrils. Films with transmittance values above 80% are typically produced by TEMPO-oxidizing CNFs with widths of 3–4 nm. Due to the dense packing of the fibrils, slow filtering, drying, and compression films are often translucent rather than optically transparent and the electrostatic forces between negatively charged particles are characterized by an underlying cholesteric pitch. CNCs exhibit a lyotropic liquid crystalline behavior in water. A phase transition from an isotropic liquid phase to a chiral nematic phase is observed from a specific concentration threshold. If the pitch in the solid films is close to the wavelength of visible light, the CNC self-organization is maintained by straightforward water evaporation, resulting in Bragg's reflection of visible light. The pH of the CNC suspension can be changed by adding electrolytes, and the energy of the ultrasound/

sonication treatment used to disperse the particles in water can be changed to produce iridescent CNC films of various colors.

Nanocellulose has been regarded as the best option to replace hard glass. In order to create flat-panel displays (FPDs), functional materials like transparent conductive films, gas barrier films, and metal wiring are often deposited on huge glass substrates before being cut into commercial sizes. However, these methods are costly and cost-effective production using continuous roll-to-roll processing is anticipated to take its place. Many requirements, including those for optical characteristics, flexibility, and thermal expansion, are met by these materials that are used to replace the glass. As a result, it is envisaged that nanocellulose-reinforced nanocomposites will be a suitable replacement for glass. The optical and electrical qualities of the flat film are two of its most crucial characteristics for electronic displays.

The cellulose nanofiber is made up of semicrystalline cellulose molecule chains aligned parallel to their axes. The hydrogen connections that bind the cellulose chains to one another laterally inside the crystalline domains provide a highly organized crystalline structure. Since the benzene rings in aramid and the glucose rings in cellulose are both bonded together instead of folding, the density and modulus of the two materials are quite comparable (Whitton 1970). When the young modulus of the perfect cellulose crystal was measured, it was around 138 GPa (Dodds and Gudder 1992) and the tensile strength was beyond 2 GPa. Yano et al. (Hayakawa et al. 2012) were the first ones to report the synthesis of transparent nanocomposite made by adding bacterial cellulose nanofibers as the reinforcing phase. Because of the enormous hydrophilic surface area, BC is made up of a network of ribbon-shaped fibrils with a width of less than 100 nm that contains around 99 wt% water. The matrix before being treated with epoxy, phenol-formaldehyde, and acrylic resin, the surplus water was squeezed out and fully dried and then cured by UV light or hot pressing. The resultant matrix usually has a fiber content of 60–70 weight percent.

Despite having a high nanofiber content, the overall light transmittance in the visible range between 500 and 800 nm was more than 80%. Less than 10 percentage points were lost in light transmittance due to the reinforcing as compared to the plain matrix resin. Transparency is drastically reduced if there is even a tiny mismatch in the refractive indices of the fibers and the matrix, which is how light transmittance is predominantly controlled in typical micro composites.

The refractive index of cellulose is 1.618 along the fiber and 1.544 in the transverse direction at 23 °C, compared to 1.483 at 587.6 nm for impregnated phenol-formaldehyde, for example. The other types of resin used in the process exhibit comparable differences. The degree of transparency due to the reinforcing element reduced lateral surface area, because of the scattering of light due to the nanofibers.

Methods for Synthesizing Nanocellulose Materials

These nanofibers offer a fantastic platform for surface functionalization through diverse approaches due to their enormous specific surface area and abundance of

hydroxyl groups inside the nanocellulose structure (Habibi 2014). The characteristics of nanocellulose discussed above provide appealing methods for selective surface modification to include practically any desired surface functionality (Hofmann and Reid 1929). Utilizing esterification processes, the hydrophobization of cellulose surfaces was accomplished in the 1920s and early 1930s and employed for food packing. Acetylation and esterification of chitin (Nair et al. 2003), starch (Jeon et al. 1999), xylan (Peng et al. 2010), cellulose nanocrystals (Nakagaito et al. 2010), cellulose fibrils (Huang et al. 2012), and cellulose fibers (Vaca-Garcia et al. 1998) have been used in a number of methods during the past few decades to make materials hydrophobic. In order to reduce moisture absorption (Sethi et al. 2018), improve interfacial affinity between nanofibers and resins (Tan et al. 2015; Trinh and Mekonnen 2018), and increase thermal stability (Sharma and Deng 2016; Qu et al. 2018), a variety of long-chain aliphatic compounds have been grafted on CNFs and CNCs using various methods. More advanced cellulose derivative-based goods have started to be taken into account as cutting-edge technology throughout time. Innovative products based on nanocellulose, such as synthetic skin, biosensors, flexible electronics, and volumetric displays, have recently received a great deal of attention. It is important to change the structure of cellulose and give it new properties in order to accomplish this purpose.

Complex multi-stage procedures such as combinations of enzymatic, mechanical, or chemical treatments are used in the extraction of pure cellulose (Rajinipriya et al. 2018). Alkali treatment (using sodium hydroxide or potassium hydroxide), bleaching (delignification with sodium chlorite or hydrogen peroxide), and acid hydrolysis (using sulphuric acid, acetic acid, or oxalic acid) are the three main phases in nanocellulose extraction processes. While CNFs are produced via a number of mechanical processes, including grinding, cryocrusting, steam explosion, microfluidation, ultrasound, homogenization, etc., CNCs are recovered by direct acid hydrolysis of pure cellulose (Khalil et al. 2012).

The four primary categories of NC extraction therapies are mechanical, pressure-induced, enzyme-assisted, and green chemical extractions. CNFs are typically created using mechanical techniques such as ball milling, twin screw extrusion, cryo crushing, etc. Crystalline forms of nanocellulose can be created using pressure-induced techniques. On both a laboratory and an industrial scale, high-pressure homogenization (HPH) is one method that is particularly effective for creating CNCs (Deepa et al. 2015).

Algae, wood, wood products, and bacteria can all be used to extract cellulose. Wood is mostly composed of cellulose microfibrils, which have cross-sectional dimensions of 20–100 nm and fiber lengths of 100–200 nm. The amorphous and crystallized components of cellulose are known to be sequentially ordered along the direction of the fiber and to divide into nanofibrils in cross-section. It is nearly impossible to separate the cellulose crystal sections due to the strong hydrogen connections that exist between its hydroxyl groups. However, the amorphous parts of cellulose are very easily broken. Therefore, natural resources must first be handled and amorphous cellulose must be broken down in order to recover NC in CNC and

CNF forms. CNC and CNF are superior to other materials in terms of tensile strength.

However, it is challenging to extract CNF and CNC from natural resources due to the fact that there are numerous resources, each with a different composition and cellulose formation. In addition to wood, other sources of cellulose include bamboo, crop wastes, discarded fruit bunches from oil palm trees, sugar cane bagasse, tunicates, and bacterial cellulose.

A well-known method for chemically and mechanically rupturing wood fibers to produce wood microfibers is called pulping.

For CNF and CNC extraction, there are two distinct methods that use mechanical, chemical, and physical processes. However, a multi-stage technique involving powerful chemical and/or mechanical methods is required to eliminate CNF and CNC.

The three substances that makeup wood fibers are lignin, hemicellulose, and cellulose. Lignin stops the wood from separating into its individual fibers. Delignifying makes sense before starting the NC extraction process. The most widely used method of lignin removal is known as kraft pulping, which entails boiling a solution made of a digester that contains sodium hydroxide and sodium sulfide.

As an intermediate source for the CNF and CNC extraction, microcrystalline cellulose (MCC), from which the majority of the lignin and sizable amounts of hemicellulose have already been removed, can be used. Acid hydrolysis can successfully dissolve the amorphous cellulose, releasing CNC into the solution.

Similar to acid treatments, cellulose enzymes can target the amorphous regions of cellulose fibers, making it easier to extract micro fibrillated cellulose. By cellulose dissolution, microcrystalline cellulose can be expanded and made more likely to separate into nanofibers. There are many other kinds of cellulose solvents, but *N,N*-dimethylacetamide (DMAc) with lithium chloride is the one that is most frequently used to dissolve cellulose (LiCl). Cellulose may be dissolved using an ionic liquid, a solvent that is environmentally friendly and recyclable. An ionic liquid containing 1-butyl-3-methylimidazolium chloride was used to dissolve and regenerate cellulose, and subsequently, it was used to make electro-active paper.

TEMPO-mediated 2,2,6,6-tetramethylpiperidine-1-oxyl radical oxidation was used to extract cellulose nanofibrils that are 3–4 nm wide and a few microns long. Even when native cellulose is subjected to TEMPO-mediated oxidation of cellulose, a regioselective surface modification of crystalline cellulose microfibrils, the original fiber morphologies are not affected.

The cellulose microfibers' inter-fibrillar hydrogen bonding needs to disintegrate under intense mechanical pressure in order for CNF to be released. Depending on the degrees and types of mechanical forces, amorphous cellulose or hemicellulose domains, a type of inter-fibrillar hydrogen bonding, can be easily broken first and inter-molecular bonding by the Van der Waals force can be broken second. The intramolecular hydrogen bonds in cellulose are extremely strong and are seldom disrupted. Amorphous domains or interfibrillar hydrogen bonds can dissolve to

release CNF. High-pressure homogenizers and grinding procedures were employed to micro-fibrillate pulp fiber.

On the other hand, the treated pulp displayed a wider distribution in fiber width. The result of combining two strategies was uniform CNF. However, the hydrophilicity of this CNF increased water retention by 545%. Several methods, such as the bead mill, pearl mill, ball mill, dis mill, and twin-screw extruder, have been tried for the extraction of CNFs. Recently, a process for extracting CNF that is ecologically benign was created: aqueous counter-collision technology (ACC). This method uses just two water jets and no additional chemical changes to transform biobased materials into nano-objects.

The capacity of cellulose nanocrystals to create a liquid crystalline phase in water at a specific concentration is another intriguing characteristic of these materials. They have a high inclination to align along one vector direction as a result of their inflexible rod-like shape (Imai et al. 1998). The nanostructures of cellulose can form nematic ordered phase which is chiral with optical properties similar to a conventional cholesteric liquid crystal when the suspensions reach the critical concentration. After the water has evaporated, the chiral nematic ordering can still be present, leaving behind iridescent films of cellulosic crystal structure (Boisset et al. 2000).

Method for Preparing Nanocellulose Composites

In contrast to the dried BC sheets, which were translucent for surface light scattering, the nanofibers are able to reduce the light scattering if the nanofibers are closely produced and the pores between the fibers are small. The nanocellulose surface must be uniformly smooth in order to improve light surface scattering. There are various techniques for smoothing nanocellulose's surface. The first technique involves laminating optically transparent polymers onto sheets of nanocellulose. Impregnation in plastics has been the second technique that has been mostly used.

The difference between the refractive indices (RI) of nanocellulose and plastic is minimized to increase the light transmittance of the nanocomposite.

Since the RI of polymers varies, the RI of nanocellulose must also be controlled. Chemical processes are used to modify the RI of nanocellulose. As a result, it is important to correctly manage CTE and the substrate's optical transparency. Acetylation is the most used type of chemical treatment. Both BC/acrylic resin and pulp that have been ground, display optical transparency in their appearances. The sheet of pulp and acrylic resin that has been homogenized, on the other hand, seems translucent. In general, nanocomposites cannot be made more transparent after 30 refiner passes; however, extra processing, such as grinding, can make materials more transparent by making them more fibrillated.

The flexibility of the BC nanocomposites, which are made of epoxy-modified siloxane and nanoparticulate boehmite, varied depending on whether BC was never dried or cured. When never-dried BC was used, the nanocomposites were studied, and the results revealed that Young's modulus and tensile strength both increased

with a decrease in elongation at the break due to the effective relationship between the boehmite-GPTS sol and the BC microfibrils; water also acts as a plasticizer.

The properties of nanocomposite containing BC are also influenced by the method of plastic introduction. The first technique of introduction is the “in situ” method, which involves adding plastics directly into the developing BC media. The second method of introduction involves “impregnating” BC into a plastic solution. The ribbon-shaped structure of nanocellulose differs from other structures in nanocomposites, according to techniques of fabricating one another.

Nanocellulose requires a pre-treatment, oxidant, or dopant to enhance its characteristics and material compatibility in order to increase the electrical properties of nanocomposites working with bacterial cellulose.

Important Factor for Optically Transparent Application

Flexible circuits have a far longer history than most people realize. Electrical conductors coated on flat substrates were taken into consideration as early as the first decade of the twentieth century for usage in radios and telephony switching for exchanging lines. Circuit innovations were sought after by the developing electronics sector for mechanized mass production. These original stiff substrates gradually developed into flexible ones, and today flexible electronics can be used in almost every electrical or electronic product where there are requirements for electrical connection of moving elements or space, shape, or restrictions. As the field of flexible electronics develops, flexible substrates will soon be utilized in screens that can be rolled up for mobility or built into clothing, as well as solar panels that can be wrapped around the exteriors of buildings.

When viewing images through the substrates, bottom-emissive displays need to have a total light transmission of more than 85% over the visible light spectrum (400–800 nm) and less than 0.7% of haze. Applying a tough coating can produce barrier and solvent resistance, whilst a scratch-resistant covering can produce a smooth surface. The material's inherent features of thermal and dimensional stability necessitate the careful selection of the plastic in order to withstand thermal cycles and high temperatures experienced during the deposition of the coating and active layers during manufacturing.

In order to turn individual pixels on or off, displays need to use a thin-film transistor (TFT) circuit that produces a regulated current source. Displays need an inorganic TFT backplane placed on the substrate for that, which means they will experience thermal stress cycles throughout the production of the panel and will thus need a substrate material with an extraordinarily low coefficient of thermal expansion (CTE). Instead of the conventional rigid glass substrates, flexible plastic substrates offer the opportunity to create strong, thin, and lightweight displays that bend, flex, and conform to different shapes. Manufacturing can also use roll-to-roll processes that are affordable. Surface smoothness, High transparency, barrier capacity, thermal solvent resistance, dimensional stability, and decreased cost are still

requirements for plastic substrates. To avoid strain and damage during the thermal cycle, the coefficient of thermal expansion is expected to be below 2×10^{-5} K.

The use of nanocellulose in flexible displays has received a lot of attention. The flat-panel display (FPD) technology is transitioning from batch processing to less costly continuous roll-to-roll. OLED displays, which use organic light-emitting diodes, are among the flat-panel screens that are now gaining prominence. Because OLED is used in larger screens for desktop monitors, laptops, tablets, and mobile devices, the OLED industry is expected to rise. By 2015, the OLED market is expected to reach \$ 5.5 million.

The traditional method involves piling up on a big substrate, which is primarily made of glass. After that, depending on the equipment, cut the panel into different sizes (Forrest 2004). As a result, the most economical method of producing glass of different sizes was the traditional method. Due to their flexibility, plastics are employed as substrates in continuous roll-to-roll processes rather than glass. Plastic's elasticity allows for easy folding and rolling up for storage when a process is not in use. As a result, the roll-to-roll method has various benefits, including as low costs, ease of distributing functional material on the substrates, and product storage (Reuss et al. 2005). There are three prerequisites to meet in order to become a substrate in a roll-to-roll process. First, the high coefficient of thermal expansion (CTE) must be less than 3 ppm K^{-1} . However, the CTE of polymers is high (approximately 50 ppm K^{-1}). Additionally, foldable or flexible plastics (around 200 ppm K^{-1}) exhibit incredibly high CTEs (Yano et al. 2005).

According to an atomistic theory of materials, the profundity of the atomic bond energy function is connected with both thermal expansion and Young's modulus, however, the relationship between the two attributes is inversely proportionate. As a result, flexible polymers with low modulus have high CTEs by nature. One option is to reinforce plastics with fibers with low CTE in order to lower CTE while maintaining flexibility. However, the refractive indices of the fibers and plastic material are precisely matched, and the presence of elements, even at micrometer levels, lowers the transparency.

Functional materials can be destroyed and damaged during the assembly and mounting process due to the difference in CTE between functional materials and plastics. Second, flexibility not only enables the gadgets to be bent or flexed but also implies cost-effectiveness. However, the plastic is delicate to tiny cracks, making it susceptible to tearing during rolling. Thirdly, in order to use in a variety of applications, high optical transparency is required.

Instead of microfibers, tinier nanofibers with lateral dimensions far shorter than the wavelength of visible light are preferable. The size of this necessary dimension should be at least one order of magnitude less than the visible light wavelength, that is, between 40 and 80 nm. Such nanofibers are already present in nature as abundant renewable resources in the form of biopolymer cellulose. This chapter will provide a brief summary of the research on cellulose nanofiber-based transparent nanocomposites, focusing in particular on efforts to limit the thermal expansion of polymeric resins for use as substrates in flexible electronics.

Coefficients of Thermal Expansion (CTE)

CTE plays a crucial role in the roll-to-roll process. The functional materials that are attached to substrates would be harmed and destroyed by the mismatch between the CTE of materials if the substrate is unable to withstand the high temperature throughout the procedure. Glass used as substrates has a CTE that is sufficient to maintain the manufacturing process's temperature, but the glass is extremely fragile and cannot be folded.

Plastic reinforcements must be used in order to increase CTE. On adding reinforcement to plastics, it lowers the CTE of plastics without loss of optical transmission. The decreased CTE associated with nanocellulose balances out the high CTEs of polymers. On the other hand, the high Young's modulus of nanocellulose is complemented by the low Young's modulus associated with plastics. Nanocomposites have different CTEs depending on different types of reinforcements, polymers, and chemical treatments. By drying mats of fibrillated kraft pulp submerged in plain acrylic resin and cured with UV light, optically transparent composites made of these materials have been created. The composite thus formed has lower CTE (17 ppm K^{-1}) (than the CTE of the pure acrylic resin. This outcome shows that the pulp's fibrillation served as reinforcement in nanocomposites (Iwamoto et al. 2005).

Bamboo nanofibers that had undergone a leaching process to remove extractives had been added as reinforcement to acrylic resin. The dried nanofiber sheet was impregnated in acrylic resin to create the nanocomposites. Bamboo nanofibers and resin were combined to create nanocomposites, which demonstrated that their CTEs were lower than those of pure resin. One of the intriguing facts is that the CTE of mature bamboo is higher than another bamboo because mature bamboo has higher lignin content (58.1 ppm K^{-1}) (Hanic and Craigie 1969).

Yano et al. (2007) investigated the relationship between the degree of crystallinity, CTE, and modulus. When the degree of crystallinity is raised, the intermolecular hydrogen bonding between the cellulose molecular chains increases. Because thermal expansion is constrained by intermolecular hydrogen bonds, a high degree of crystallinity denotes a low CTE. Increased grinding causes the deterioration and disintegration of fibrillated fibers when more passes are made through the grinder. In conclusion, CTEs and material modulus are influenced by the degree of crystallinity. Furthermore, because cellulose contains nanofibers that are connected by hydrogen bonds between intermolecular, it is difficult to separate these nanofibers using mechanical processes (such as grinding, homogenizing, etc.).

Fukuzumi et al. (2009) transformed the majority of the hydroxyl groups of cellulose into negatively charged sodium carboxylate groups using 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) in order to split and divide into microfibril without destruction and damage. Then using a blender-style homogenizer, they produced TEMPO-oxidized cellulose nanofiber (TOCN). The CTE of TOCN (2.7 ppm K^{-1}) is lower than glass (about 9 ppm K^{-1}) compared with silicon (3 ppm K^{-1}).

Excessive acetylation causes negative effects on the CTE of nanocomposites including bacterial nanocellulose. The hydrogen bonding between the cellulose molecular chains is weakened by replacing the hydroxyl groups of BC with acetyl groups from acetylation. The nanocomposite of acetylated bacterial nanocellulose undergoes thermal expansion readily (Ifuku et al. 2007). If BC is used as a reinforcement, the coefficient of thermal expansion is affected by layer direction within BC. The rationale for the layers is that only bacteria near the surface of the culture medium can maintain their activity due to cell development. As a result of the strong hydrogen bonding between nanofibers made up of dense and finely branched fibers, it was discovered that the CTEs of plane direction for BC nanocomposites was 4 ppm K^{-1} . On the other hand, it was discovered that the CTEs of thickness direction for BC nanocomposites were 555 ppm K^{-1} (Nogi and Yano 2008).

When bacterial nanocellulose is added to the epoxy material, the CTE of the composite is reduced drastically from $6 \times 10^{-6} \text{ K}^{-1}$ to $120 \times 10^{-6} \text{ K}^{-1}$. The nanocomposite of BC/phenol-formaldehyde was smaller at $3 \times 10^{-6} \text{ K}^{-1}$. The modulus was around 20 and 21 GPa, while the tensile strength was 325 MPa for the nanocellulose composite. BC nanocomposites are flexible, light, and very transparent despite having a high modulus. Nogi et al. (Morand and Briand 1996) assessed how the refractive index of the matrix acrylic resin affected the transparency of nanocomposites of bacterial nanocellulose. The total and regular transmittances at a wavelength of 590 nm were greater than 85% and 75%, respectively, while the resin's refractive index ranged from 1.492 to 1.636. Similarly, when the temperature changed from room temperature to 80°C , the matrix refractive index changed by 0.014, while the regular transmittance of composites remained constant (Higgins et al. 2008).

The transmittance of resin reinforced by BC decreases linearly with fiber content; however at the fiber content of 66 wt%, the loss from neat resin to composite is only 13.7 percentage points. The CTE of acrylic resin is reduced from $86 \times 10^{-6} \text{ K}^{-1}$ for the neat acrylic resin to $15 \times 10^{-6} \text{ K}^{-1}$ and $10 \times 10^{-6} \text{ K}^{-1}$ with the addition of BC nanofiber at 30 wt% fiber content, and 50 wt% fiber content.

The high moisture content of nanocellulose, caused by the presence of hydroxyl groups in its molecular chains, makes cellulose-based products very hydrophilic. Nogi et al. and Ifuku et al. (Atalla and Vanderhart 1984) acetylated the BC nanofibers, substituting acetyl groups for the hydroxyl groups on the surface of the fibers, to decrease the sensitivity of moisture in BC nanocomposite. The water content of the untreated BC composite at 60 wt% fiber content was 3.12% when exposed to a relative humidity of 55% at ambient temperature, while the moisture content of the acetylated nanocomposite of BC was decreased to 1.33% at a fiber load of 66 wt%.

Young's modulus was decreased from 23.1 to 17.3 GPa, and the CTE of the BC sheet improved from $3 \times 10^{-6} \text{ K}^{-1}$ of the untreated to merely $0.8 \times 10^{-6} \text{ K}^{-1}$ of the acetylated sheet. The less hydrophilic acetylated BC's improved compatibility with the hydrophobic matrix may be the cause of the decreased CTE. The acetylated BC had transparency in the region of wavelength around 400 nm while maintaining visibility in the other portions of the spectrum, according to the regular transmittance in one of the BC/acrylic resin combinations.

The neat BC sheet is tightly packed, as seen in the SEM images, whereas the acetylated BC sheet is separated from one another. This suggests that the acetyl groups present on the surface of fibrils prevented their aggregation through hydrogen bonds, maintaining the uniformity of the individualized nanosized lateral dimensions of the nanofibers. The BC nanocomposite that is acetylated showed a lesser decline in transmittance than the untreated BC nanocomposite when both were exposed to a temperature of 200 °C for several hours, indicating that they resisted thermal degradation along with an increase in optical transparency.

BC was used with a translucent resin (VanderHart and Atalla 1984) which has a low Young's modulus to reduce the thermal expansion of composites, particularly at a fiber concentration as low as 5 wt%. Due to the resin's low modulus of only 25 MPa, the resultant composite has a modulus in the range of high-density polyethylene at 355 MPa. Due to its low modulus, the composite was incredibly flexible and even foldable without suffering any harm. The in-plane CTE was exceptionally low at just $4 \times 10^{-6} \text{ K}^{-1}$, despite the low modulus.

For materials with high Young moduli will have lower CTE which can be explained by the particular structure of BC pellicles, which consists of many layers of nanofiber networks that create strong in-plane networks which are poorly coupled along the thickness direction of the pellicles. The material expands its thickness, even at low concentrations, fibers are able to restrict the minor stresses produced by the expansion of the low modulus matrix in the in-plane direction. Although the in-plane CTE is extremely low due to its anisotropic behavior, the material has increased flexibility and can be folded more easily.

Optical Transparent

The nanofibers are dense and the pores in the fibers are small which suppress the light scattering property while the bacterial nanocellulose is translucent for surface light scattering. Nanocellulose is treated with emery paper to increase transparency. The nanocellulose surface must be uniformly smooth in order to improve light surface scattering. There are various techniques for smoothing nanocellulose's surface. The first technique involves laminating optically transparent polymers onto sheets of nanocellulose. Impregnation in plastics has been the second method that has been mostly used.

The optical transparency of nanocomposites made of nanocellulose and plastics is increased by the fact that the diameter of nanocellulose is smaller than the wavelength of visible light. The difference between the refractive indices (RI) of nanocellulose and plastic is minimized to increase the light transmittance of the nanocomposite. Chemical processing changes the RI of nanocellulose. As a result, it is important to carefully manage CTE and substrate optical transparency and acetylation is the most popular type of chemical treatment (Nogi et al. 2009).

Additionally, nanocomposites containing BC that had high DS displayed excellent transmittance because the RI of the BC dropped with increasing DS. Additionally, due to the improved bond that acetylation creates between

cellulose and polymers, BC that had been acetylated was successfully impregnated (Nakagaito et al. 2010). The insertion of bulky acetyl groups into nanocellulose led the thickness of the material to thicken, which allowed the thickened nanocellulose to interact with transmittance. Furthermore, excessively acetylated nanocellulose's RI would be lower than that of plastics.

In order to create opaque composites, bacterial cellulose (BC) seems to be a good source of nanofibers, however, the fermentation techniques used to make BC are incredibly sluggish. The more accessible wood or plant fibers might be used as a substitute, but first, the nanofibers must be removed. In a very intricate structure, cellulose nanofibers are discovered embedded in a matrix of hemicelluloses and lignin in the fiber cell wall of plants. In order to thoroughly reduce the size of the filament bundles and fibrillate the wood pulp fibers.

Iwamoto et al. (Horii et al. 1987) began with a cellulose shape known as micro fibrillated cellulose (MFC). He added an extra grinding process to the phases of refining and high-pressure homogenization. MFC produced by 14 homogenizer passes had a wide range of fibril widths, ranging from a few tens of nanometres to a few micrometers, which results in composites that are much less transparent than BC-based composites. The thicker fibril components impacts light scattering, which produces lower transparency. The 10 passes through the grinder added to the 14 passes through the MFC homogenizer to rise to fibril bunches that ranged in width from 50 to 100 nm. This MFC that had been ground into fibrillation was dissolved in water, and the suspension was vacuum filtered to create a thin sheet. Sheets were oven dried, coated with acrylic resin, and cured by UV radiation, and the light transmittance of grinder-fibrillated fiber/acrylic resin composite, pure acrylic resin, BC acrylic resin, and homogenizer-fibrillated fiber/acrylic resin was measured. The measured young modulus was lower than the modulus of BC composites, 7 GPa which means a more flexible substrate. The coefficient of thermal expansion for the matrix was 17×10^{-6} K from 86×10^{-6} K of neat resin.

Abe et al. (2007) created a new methodology that produced consistent nanofibers which has a diameter of 15 nm by only one run through the grinder to limit damage to the nanofibers in order to extract significantly greater nanofibers by grinding. Iwamoto et al. (2007) earlier investigated and analyzed the resulting nanocomposites' mechanical characteristics to determine how severe the mechanical shear that the grinder imparted to fibrillate was. Although composites were more transparent after five runs through the grinder and beyond, the morphology of the nanofibers had not changed, nor had their transparency. However, due to a decrease in the degree of polymerization and crystallization, its thermal expansion and tensile capabilities were severely diminished. It was also made clear how hemicelluloses affect nano fibrillation (Iwamoto et al. 2008). Hemicelluloses prevent the cellulose microfibrils from forming irreversible hydrogen bonds when pulp fibers are dried. Hemicelluloses are also plasticized by absorbing water when they are rewetted, which facilitates posterior nano-fibrillation. After chemically removing lignin and hemicelluloses, Abe decided to leave the fiber in a water-swollen state rather than dry it, which would have produced strong hydrogen bonds between the nanofibers. Later, Okahisa et al. (2009) created an organic light-emitting diode (OLED) layer on

top of a low Young's modulus resin-based optically transparent film, cellulose nanofiber was used as the reinforcing material which was produced from wood. Saito et al. (2006) discovered an even easier method of chemically extracting cellulose nanofibers. The C6 hydroxyl groups on the surface of the cellulose were changed into negatively charged sodium carboxylate groups by oxidizing the surface of the nanofibers using a 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) radical catalyzed method. The nanofibers' mutual adhesion was relaxed by electrostatic repulsion, and mechanical abrasion and agitation by a Waring blender were sufficient to separate the original fibers into nanofibers with diameters of 3–5 nm. The films produced by filtering of these nanofibers had transmittances exceeding 85% over the visible light spectrum and were optically clear (Saito et al. 2007). The films' tensile strength was comparable to that of Nogi et al. (2009) cellulose's nanofiber paper, but their coefficient of thermal expansion was considerably less, 2.7×10^{-6} K even though their Young's modulus was only about half that of Nogi's paper. Additionally, the films showed strong oxygen-barrier characteristics. Only three percentage points were lost in the light transmittance of the composite at 600 nm compared with 89% of neat resin when nanofibers were combined with polystyrene resin (Fujisawa et al. 2012) at a 10-weight percent fiber load. The coefficient of thermal expansion of neat fiber was 116.7×10^{-6} K to 63.7×10^{-6} K for the composite. The reduction of the coefficient of thermal expansion was similar to the values that were reported by Nogi et al. (2006), in his work on bacterial nanocellulose which was reinforced at a comparable fiber content by acrylic resin. These fibers that obtained the TEMPO oxidation method are finer and were produced by less energy-intensive extraction than mechanical methods and extracted more quickly.

Producing nanofibers using electrospinning is an alternate way to get around the pricey extraction process. In order to create continuous fibers with a nanometre diameter, Liu and Hsieh (2002) spun cellulose acetate using a solvent mixture of *N*, *N*-dimethylacetamide (DMAc), and acetone. To create cellulose nanofibers, these cellulose acetate nanofibers underwent further processing to hydrolyse the acetyl groups and restored the hydroxyl groups. Using polyvinyl alcohol (PVA) as the matrix resin, composites were created, and optically transparent films were produced (Tang and Liu 2008). PVA was fortified with cellulose acetate nanofibers, regenerated cellulose nanofibers with diameters between 250 and 940 nm, as well as nanofibers with a lower diameter and a narrower range between 160 and 290 nm, in order to examine the factors impacting transparency (Tang et al. 2011). The transmittance of the plain PVA was around 92% in the center of the visible spectrum. In the cellulose acetate nanofiber composite, the transmittance was about 80% at 20 weight percent and just 18% at 50 weight percent fiber load. The transmittance of the regenerated cellulose nanofibrous counterpart had the same diameter, which was over 85% at fiber quantities up to 30 weight percent and dropped to 38% at 50 weight percent. In contrast, the smaller regenerated cellulose nanofiber reinforced PVA films showed transmittance of 90% for fiber loads up to 30 wt%, remaining in the 85–88% range at 40 wt% fiber content, and reaching 55% with a 60% weight percentage of fiber. This research underlined the importance of fiber size while also highlighting their interfacial interaction with the matrix resin. Since cellulose acetates are less

compatible with hydrophilic PVA than reconstructed cellulose, they are more hydrophobic. Stronger fiber matrix adherence of cellulose-PVA interfaces results in progressive fluctuation in optical characteristic impedance, which improves transparency by reducing light reflections and refraction.

Later, a paper lacking matrix resins was produced that was optically transparent. It was given the name “cellulose nanofiber paper” by Nogi et al. (2009) and is constructed entirely of the same chemical components as regular paper, with the exception of a physical variation in the size of the fibers, which were produced using a process created by Abe et al. (2007). The morphology of cellulose is the only aspect of the paper that differs from standard paper. After filtering and drying, a nanofiber aqueous suspension was transformed into paper with a density of 1.53 g/cm^3 , which is quite similar to the 1.59 g/cm^3 of the cellulose crystallite (Sugiyama et al. 1991). Scanning electron microscopy measurements supported the virtually complete elimination of the tiny interfibrillar cavities. This paper was initially only translucent, but after meticulous polishing reduced the irregularity of its surfaces, the transparency increased to 71.6% at 600 nm wavelength. Along with having a tensile property of 13 GPa and a strength of 223 MPa, the thermal expansion coefficient of $8.5 \times 10^{-6} \text{ K}$ was comparable to that of glass. In order to smooth up the interfaces of the cellulose nanofiber sheet, the authors also coated it with clear polymers (Nogi and Yano 2009) and proposed that continuous roll-to-roll production techniques might be used.

Flexibility

Nanocellulose substrates' versatility has various benefits, including ease of processing and storage. Low modulus and high elongation at the break of nanocellulose are shown by the foldable and flexible substrates which demonstrate the flexibility of the nanocomposites that plastics provide. Pure acrylic resin has a very high elongation at break, yet the sheet tears when folded since the substance is sensitive to even the smallest fissures. Nanocellulose (BC) stops the spread of cracks in nanocomposites, allowing the materials to fold without cracking.

The flexibility and mechanical properties of nanocomposite depend on the dried and never dried BC. The flexibility of BC nanocomposite composed of nanoparticulate boehmite and epoxy-modified siloxane changes with the BC used never dried and dried state (Barud et al. 2012).

When utilized in dry BC (Barud et al. 2012), however, the elongation at break increases with a decrease in tensile strength and an increase in Young's modulus. The properties of nanocomposite containing BC are also influenced by the method of plastic introduction. The first technique of introduction is the “in situ” method, which involves adding plastics directly into the developing BC media. The second method of introduction involves “impregnating” BC into a plastic solution. The ribbon-shaped structure of nanocellulose varies depending on the process for creating nanocomposites.

Mechanical Strength

The hydrogen bonding between the nanofibers was broken off when PVA was coated on the surface of the BC, which increased the elongation at break when compared to pure BC. Young's modulus and yield strain in the case of MFC is influenced by the number of passes through the grinder. Young's modulus and yield strain decrease with more passes made. These findings suggested a connection between intermolecular hydrogen bonding and fibrillated fibers with pass number. The shape of the fiber was the source of the decrease in yield strain. First, additional grinder treatments led to the breakdown of the fibrillated fibers while also boosting hydrogen bonding. Second, the aspect ratio of the fiber fell as a result of the shear force that reduced the fiber's length.

Optical Application of Nanocellulose

For material-specific goals, photonic applications of nanocellulose are appealing. First, CNCs' liquid crystalline behavior produces opalescent films with specific optical properties; second, both CNFs and CNCs are capable of producing independent optically clear films.

The cellulose's structure and surface chemistry lend these materials their flexibility; with little effort, nanocellulose may be made compatible with both hydrophobic and hydrophilic substances, employed as a host for optically active nanoparticles, and modified to take covalently bound molecules. CNCs may easily create chiral films that are colored, nematic, and iridescent by evaporating aqueous solutions (Schlesinger et al. 2015).

The chiral nematic structure of CNCs is frequently left-handed because of the right-handed nanocellulose chirality, whereas liquid crystals formed from cellulose derivatives exhibit either right-handed or left-handed chirality. Therefore, when the helical pitching is in the order of the visible light wavelength, the chiral nematic CNC films selectively reflect left-handed light and appear colorful. Applications for such systems could be in chiral plasmonic, soft actuators, sensitive hydrogels, optical filters, anti-reflective coatings, and flexible electronics, and for chemical sensing CNC-templated composite which is mesoporous and has a fluorescent organosilica film.

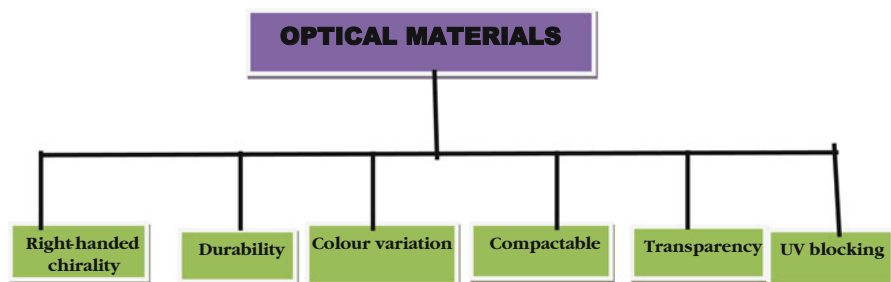
Furthermore, the modification of film color is rather simple because the helical pitch is sensitive to a range of factors. The spectacular photonic colors present in nature, such as those in butterfly wings and the seed hulls of the *Margaritaria nobilis* fruit, are comparable to the phenomenon of structure color. Over the past five years, a wide range of left-hand chiral materials, including cellulose, nanocrystalline, mesoporous silica, carbon, titanium, and organosilica as well as polymer/CNC nanocomposites, have been accessed via CNC templating. The overall strategy is to synthesize chiral nematic complexes from CNCs and materials of interest, often silica or polymers, and then either remove one element selectively or employ the component as part of nanocomposites.

Biomaterials are created by removing a portion of the basic nanocomposite to form a chiral highly porous substance that could contain components or serve as a

rigid template to create further chiral materials. Other CNC-based systems with varied optical characteristics, such as surface plasmons, UV blocking, fluorescence, and low refractive index, have been created in order to contribute to chiral nematic materials (Habibi et al. 2010). Greenhouse plastics, particle tracking, sensing, and anticounterfeiting technologies are some of the applications that have been suggested. ZnO absorbs UV light, therefore covering polyethylene with a combination of ZnO, SiO₂, and CNCs not only strengthened the plastic but also protected it against dangerous UV rays. Infrared blocking was also accomplished by the SiO₂ nanoparticles, which helped with energy conservation at night. CNF films are crucial as substrates for optoelectronic devices as well as in areas of packaging and coating due to their optical clarity and robust mechanical qualities (Haywood and Davis 2017).

CNF films employ additives like additional nanomaterials, polymers, or surface changes to add desirable qualities like hydrophobicity, similar to CNC-based systems. The drawback of CNFs and CNCs is their relatively high viscosity, even at trace levels preventing high-speed coating from using them. Given that the thickness of the NFC is less than the wavelength of visible light, it possesses good optical characteristics. Nanofibril-prepared paper is transparent as a result. This can be included in a number of applications, including those involving sensors, solar panels, and electronics. Jia Huang and colleagues documented the flexible field effect transistors that are imprinted on nano paper for use in green electronic transistor applications (Huang et al. 2013).

Numerous NC-based sensing devices demonstrated openness to various inputs. NC is frequently changed with different interfacial groups, other polymer chains, or even other nanoparticles to get a variety of properties and functionalities along its surface in order to create low-cost smart materials that are sensitive to biotechnology. The capacity to mix different functional nanomaterials with nano-scaled cellulose nanoparticles in various configurations opens up a wide range of possibilities for their future use in electrochemical, electrical, and optical devices. This recently developed nano cellulosic material proved a very adaptable choice for numerous sensing system application domains. According to Kan et al., a system based on the dispersion of pH-sensitive polyvinyl pyridine grafted CNC involving particle aggregation at basic pH was effective in biological applications for therapeutic release as well as industrial separation approaches. Additionally, NC has been employed as a support for conductive materials, fluorescent dots, or plasmonic, or catalysts. Here, new sensing platforms that induce sensing responses to chemical and physical inputs are identified (Soriano and Dueñas-Mas 2018).



Safety, coatings, and cosmetics labels can all use the eye-catching iridescent color of CNC self-assembly films. The visual quality of the films is immediately impacted by manipulating the direction of the cholesteric structure using external sources like magnetic fields, electric fields, or shear pressure. For instance, under various magnetic fields, the resulting CNC films exhibit a distinct color variation. The ability to use CNC self-assembly in film optics is made possible by the adjustable visual appearance. CNC films can be utilized as a color-controllable iridescent pigment. However, the sulfate ester groups on the surface of CNC limit the use of CNC films in pigments by making them prone to heat degradation and redispersion in water.

Thus, the pigment utilized in this CNC film can be applied to a variety of industries, including packaging for food, medicine, and cosmetics. Additionally, according to Zhang et al., CNC films can have some safety features by carefully regulating the use of the optical brightener (OBA) TINOPAL, which is used to modify the P and zeta potential. Public encrypting as an anti-counterfeiting strategy has become a feasible prospect. The safety elements include overt security (selected circular reflections and fluorescence) and covert security (iridescence).

A polysaccharide found in nature called cellulose has thousands of connected D-glucose monomer units. The primary structural element of the cell wall of plant cells is a natural polymeric type (pseudo-plastic) substance. In contrast, nanocellulose is a variety of forms of cellulose that has a nanostructure. Nanocellulose can be found in three different varieties. These are bacterial nanocellulose, micro/nano-fibrillated cellulose, and cellulose nanocrystals. The most often employed of these are cellulose nanocrystals and fibrillated nano cellulose structures, particularly in the creation of optical films.

Compared to bacterial nanocellulose, these two types offer a wide range of advantageous qualities, and they are much simpler to create in large quantities, thus they are employed more frequently. The characteristics of nanocellulose structures include high chemical stability, thixotropy, effective electronic and optical properties, high tensile strength (up to 500 MPa), high stiffness and strain, excellent barrier properties, no toxicity, and the capacity to self-assemble when deposited (i.e., through coating methods).

Optical Films

Nanocellulose-based films are well known for having optical transparency (i.e., low light scattering) as well as a variety of unusual optical characteristics, including iridescence, selective left circularly polarized (LCP) light reflection, and selective right circularly polarized (RCP) light transmission. They have been used in a variety of optical applications because of these characteristics, which are complemented by their high strength and wide surface area.

Additionally, nanocellulose is known (in certain conformations) to exhibit a birefringence through an optical anisotropy caused by the orientation of the nanocellulose structures during the self-assembly process when it forms multi-layer, multi-component films.

As optical filters, cellulose nanocrystals can self-assemble into three-layer sandwiched films with nanocellulose layers that are each in a separate liquid-crystal phase. These manufactured films are made of helicoidal cholesteric reflector materials sandwiched between a nematic-phase retardant layer.

The layered structure and various conformations of the nanocellulose replicate the polarization-insensitive reflectors discovered in the wings of *Plusiotis resplendent* beetles. These optical films are well recognized for having a high reflectivity that can be adjusted for both the visible and near-infrared spectrums. As a result, these optical filters may have a variety of uses, such as color filters, smart clothing, and solar gain regulation in building technologies.

Architectural windows, car windshields for navigating, and safety systems are just a few examples of vision-area applications where transparent nanocomposites used as OLED substrates can be used. Head-up display (HUD) navigation has recently begun to use this property in cars. Additionally, the adaptability of nanocomposites offers the possibility of using mobile phones, PDAs, tablet PCs, and sewing computer chips, cell phones, and GPS receivers into clothing. Additionally, transparent nanocomposites used as OLED substrates can be elastic and bendable, making it possible to develop flexible displays employing nanocomposites (Schultz et al. 1983). The current manufacturing process has to be enhanced for use as an industrial product. A fracture or other flaw might appear on a substrate's surface as a result of poor nanocomposite fabrication. Water and air contamination can also readily harm a display's surface (Dupeyre and Vignon 1998). Some of these issues are solvable. Fibrous cellulose can be manufactured by covering plastics with the substance. The manufacturing methods and their enhancements do not need additional funding because they can be produced using industry-standard technology. Depending on the conductance of the conductive polymer, electromagnetic shielding can be applied to optical equipment, display systems, sensing, batteries, etc., (Beg et al. 2000) easily by using transparent nanocomposites as packing material. Additionally, flexible, iridescently regulated, and multi-stimuli-sensitive optical films can be produced using cellulose nanocrystals. These nanocellulose films, which function as a composite material, have exceptional mechanical robustness, allowing them to be employed as free-standing iridescent photonic films with tuneable structural colors. They can be used to identify environmental changes like mechanical compression and humidity. They alter their color in response to outside stimulation to show that something has changed in the surrounding environment. They can be used repeatedly in various settings because the color change is reversible. These optical films may be used as decorative coatings, colorimetric sensors, and anti-counterfeiting technology.

The usage of ZnS-sodium carboxymethyl cellulose composite sheets as a sort of security paper for optical signatures is another potential use for cellulose. The quantity of ZnS-blende nanoparticles present in the composite affects its optical properties, particularly its optical transparency, which is highly tunable. Depending on how many nanoparticles are placed into the cellulose-based film, the optical transparency of such films can range from 50% to 90%. In contrast to many other optical applications, this one is less developed; therefore it might take some time before the potential benefits are realized.

For material-specific goals, photonic applications of nanocellulose are appealing. First, CNCs' liquid crystalline behavior produces opalescent films with specific optical properties; second, both CNFs and CNCs are capable of producing optically clear, independent films.

The cellulose's structure and surface chemistry lend these materials their flexibility; with little effort, nanocellulose may be made compatible with both hydrophobic and hydrophilic substances, employed as a host for optically active nanoparticles, and modified to take covalently bound molecules. Schlesinger et al. 2015 developed CNCs which may easily create chiral films that are colored, nematic, and iridescent by evaporating aqueous solutions (Schlesinger et al. 2015).

The chiral nematic structure of the CNCs is frequently left-handed due to the underlying right-hand nanocellulose chirality. Liquid crystals formed from cellulose derivatives exhibit either right-hand or left-hand chirality. Therefore, when the helical pitch is in the order of the visible light wavelength, the chiral nematic CNC films selectively reflect left-handed light and appear colorful.

Furthermore, the modulation of film color is rather simple because the helical pitch is sensitive to a range of factors. The spectacular photonic colors present in nature, such as those in butterfly wings and the seed hulls of the *Margaritaria nobilis* fruit, are comparable to the phenomenon of structure color. Over the past five years, a wide range of left-hand chiral materials, including cellulose, mesoporous silica, nanocrystalline titanium, carbon, and organosilica, as well as polymer/CNC nanocomposites, have been accessed via CNC-templating. The overall strategy is to synthesize chiral nematic compounds from CNCs and materials of interest, usually polymers or silica, and then either remove one element selectively or employ the component as part of nanocomposites.

A chiral mesoporous material that can host molecules or act as a hard template to create other chiral, mesoporous materials is produced by the selective removal of a section of the initial nanocomposite.

Potential uses for these systems include soft actuators, sensitive hydrogels, anti-reflective coatings, optical filters, flexible electronics, and chiral plasmonic. One example is a CNC-templated, mesoporous, fluorescent organosilica film for chemical sensing. Habibi et al. (2010) developed other CNC-based systems with other optical properties, such as UV blocking, fluorescence, surface plasmons, and low refractive index that have been created in order to contribute to chiral nematic materials (Habibi et al. 2010).

Greenhouse plastics, particle tracking, sensing, and anticounterfeiting technologies are some of the applications that have been suggested. ZnO absorbs UV light, therefore covering polyethylene with a combination of ZnO, SiO₂, and CNCs not only strengthened the plastic but also protected it against dangerous UV rays. Infrared blocking was also accomplished by the SiO₂ nanoparticles, which helped with energy conservation at night. Finally, CNF films are crucial as substrates for coatings and packaging as well as for applications like optoelectronics due to their superior mechanical qualities and optical clarity.

To learn more about the interconnected impacts of liquid crystalline phase behavior, flow alignment, and microstructural relaxation on cellulose nanocrystal

(CNC) films' alignment and optical properties, rheo-optics, micro spectrophotometry, and optical contrast studies were used. Cross-polarized microscopy pictures can be used to directly determine changes in anisotropy, and optical contrast measurements have been demonstrated to be a reliable and simple method for doing so. The anisotropy of dried CNC films as well as the microstructural relaxation following the cessation of shear were both constantly measured using this technique. Compared to isotropic or biphasic dispersions, aqueous liquid crystalline CNC dispersions show better alignment following shear. However, lower alignment was seen in CNC gels at equivalent shear (Haywood and Davis 2017).

CNF films integrate additives like additional nanomaterials, polymers, or surface changes to add desirable qualities like hydrophobicity, similar to CNC-based systems. The downside of CNFs and CNCs is their relatively high viscosity, even at low concentrations, which prevents them from being used for high-speed coating.

Due to its remarkable optical properties, nano-fibrillated cellulose has a thickness that is smaller than the wavelength of visible light. Nanofibril-prepared paper is transparent as a result. This can be included in a number of applications, including those involving sensors, solar panels, and electronics. Jia Huang et al. documented the flexible field effect transistors that are produced on nano paper for use in green electronic transistor applications.

The self-ordering nature of cellulose nanocrystals, like that of many polysaccharides, results in a variety of intriguing optical features. More than 20 years ago, it was discovered that films made of cellulose and cellulose derivatives kept ordered chiral nematic phases (Liao et al. 2006; Li et al. 2009). Gray and colleagues also discovered that when cellulose nanocrystal films were hardened by evaporation, they generated "helical structures mimicking those in many biological skeletal systems" (Liebert and Heinze 2008). Helicoidally arranged cellulose nanocrystal sheets that reflect polarized light are described by Revol et al. The visible, ir, and uv spectrums of the light reflected by these films were said to be controlled (Lisperguer et al. 2009; Lin et al. 2010). According to reports, the colors shift with viewing angles, making them appropriate for security papers and many applications.

Recently developed nano cellulosic material proved a very adaptable choice for numerous sensing system application domains. In a system based on dispersed pH-sensitive polyvinyl pyridine-grafted CNC involving flocculation at basic pH, Kan et al. stated that it has assured techniques of industrial and biomedical separation as well as in the biomedical application for therapeutic release. Additionally, NC has been employed as a support for conductive materials, plasmonic or fluorescent dots, or catalysts.

Ouyang et al. (2013) developed energy storage, triblock-copolymer ion gels, carbon nanotubes, and bacterial nanocellulose sheets have been proposed as supercapacitors.

Other than BNC, sources of nanocellulose have been obtained from plant materials such as kraft pulp, birch pulp, and sugarcane bagasse. Research on the development of CNC implementations in electro-active materials, such as electrically conductive materials, dielectric materials, microelectronic components, etc. is now underway and will likely include certain pertinent materials, such as proteins,

starches, and peptides. The significance of CNCs in electrical applications is primarily attributable to their adaptability, piezoelectric and dielectric capabilities, and durability characteristics that are comparable to those of other bio-derived materials.

CNC composite films are used for a variety of optical purposes. These composite materials, which also contain additional components, are frequently employed in security features, micro-optical devices, polarizers, and other things. The CNC/PEG thin film developed by Gu et al. (Demirci et al. 2014) was clearly iridescent, had good mechanical properties, and had great adhesion. Kumacheva and his co-workers (Schyrer et al. 2014) prepared a CNC composite by adding latex nanoparticle which has close-to-uniform fluorescence, circular dichroism, and birefringence. In order for the films to have the ability to prepare polarizers, the nanoparticles can alter the P of the liquid crystal and form CNC cholesteric structures on their own.

Khan et al. (Al-Homoud 2005) added phenol-formaldehyde resins to CNC self-assembly in order to create polymer composites that were guided by CNC self-assembly. Films that were porous were produced when the CNC was removed. Potential images may arise as the film swells by depositing acid or formaldehyde in various areas of the film. Text and images can be printed using inkjet technology as higher-resolution photonic patterns, making this material suitable for use as a security material.

Similar to this, Schlesinger et al. (Moon et al. 2011) generated CNC/SiO₂ composites using the EISA method by using a CNC/TMOS system, and then they removed the CNC and added gold NRs. A controllable cholesteric structure was produced as a result of the interplay between the surface plasmon resonance of CNC chiral nematic and gold NRs assembly. Security devices would be another area where this material has a lot of potential applications. Mark et al. (Eichhorn et al. 2001) used the reverse emulsion approach to grow CNC tactoid in water microdroplets to create the cholesteric structure of CNC in polymeric microspheres. They applied this technique to silica microspheres containing cholesteric structures, resulting in materials with a mesoporosity and large surface area which might be used to create micro-optical devices.

Conclusion

Many nanofibers are available in the nature, but cellulose is one of the remarkably useful nanocomposites which can be extracted from plant fiber in stems and leaves, the trunk of the tree, tunicates, bacterial cellulose, and so on. The cellulose is then used to make new composites with different properties with distinctive characteristics for uses not anticipated by their natural evolution. Cellulose has the chance to once again act as the reinforcing phase of transparent plastics for flexible electronic-based products, as it did with the common paper that once served our requirements. Electronic products frequently include flexible circuit boards, and soon the substrate of image displays will be made of flexible materials as well. The nanocellulose reinforcement significantly advances the development of film-display technology, as it has been emphasized throughout an entire chapter on the significance of

nanocellulose in optical applications, particularly electronic displays. This is due to the material's excellent optical and electrical conductivity properties. Additionally, there is a strong likelihood that the market will expand in the not-too-distant future, ensuring this material's bright future. It is to be assumed that the importance of nanocellulose composite has played a significant influence in the development of applications for electronic equipment. The fundamental research that has already been accomplished will serve as the foundation for more novel materials, whether or not this promise is realized commercially.

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Biopolymers for Edible Films and Coatings in Food Applications

38

Maria Râpă and Elisabeta Elena Popa

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Abstract

Nowadays, there is an increased interest for developing of innovative edible food packaging, prepared as thin layer and then used as a wrapper on food materials, or edible food coatings adhering to the food surface. The essential benefits of edible food packaging systems, by comparison with petroleum-based food packaging materials, are the following: They represent an entire component of the food product, have a role in extending shelf-life, can be eaten without having to take out and discard the package, decay easily, and are environmentally friendly. The aim of this chapter is to investigate the advantages and limitation of the main biopolymers used for fabrication of edible and coating food packaging, their

M. Râpă (✉)

Faculty of Materials Science and Engineering, University POLITEHNICA of Bucharest, Bucharest, Romania

e-mail: maria.rapa@upb.ro

E. E. Popa

Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine Bucharest, Bucharest, Romania

compositions with additives, plasticizers, and bioactive agents, as well as the performance required for use in food packaging applications.

Keywords

Biopolymer · Edible film · Coating · Food application

Introduction

Global plastics production worldwide increased from 2015 to 2020 to 367 million metric tons, by roughly 3%. The aforementioned data shows the development of plastic manufacturing, with strong outcomes for the ecosystem and human health. Among this, food packaging is the most important sector, which creates packaging needed for our life, from bags to food containers. In 2015, the income generated by the food packaging domain was US\$ 839 billion, and up to 2020, the annual percentage growth rate was 3.5%. The main roles of food packaging are to keep food safe from chemical, physical, biological, and environmental contaminants (Theagarajan et al. 2019). Recently, the consumption of single-use plastics has gained an increase attention due to the generation of huge amount of waste. Plastics waste increased from 1% in 1960 to >10% by 2005. Data shows that every year, 5–13 million tons of plastics accounting 1.5–4% of global plastics production have ended up in the oceans [1]. It was estimated that from all plastic waste generated 11% are incinerated and only 19% are recycled (Buch et al. 2021). The initial strategy applied to plastic manufacturing was changed from “produce, use and throw away,” characteristic to linear economy toward the circular economy based on the closed-loop process through recycling, reduction, reuse, and recovery (Lombardi et al. 2021; Knappe and Schmidt 2021).

The most known materials used for food packaging materials are those obtained from petrochemical sources, such as poly(ethylene) (PE), poly(ethylene terephthalate) (PET), poly(propylene) (PP), and poly(styrene) (PS). These polymers represent optimal matrices for fabrication of food packaging in safe conditions and conform to the national and international regulations. Despite these advantages, conventional packaging materials raise a huge problem because of long persistence in the environment caused by the lack of biodegradability (Sid et al. 2021). In this context, the optimal conditions to minimize the impact of food packaging waste are to build biodegradable and edible food packaging, with low impact on environmental and human health. Thus, last time, scientists, scholars, customers, governments, and other interest groups were searching for sustainable and green materials to produce biodegradable packages to clarify the expanding issue of plastic waste disposal. In 2019, the global market value for biodegradable plastic packaging showed around \$ 4.7 billion. By 2025, it is predicted to increase approximately US\$ 12.1 billion (Mahmud et al. 2021). Biopolymers such as poly(lactic acid) (PLA), poly

(hydroxyalkanoates) (PHA), chitosan (CS), starch, and cellulose are used intensively in the food packaging field due to their environmental advantages regarding the global carbon footprint and waste decreasing. The biopolymers possess many advantages as compared with conventional polymers used for food packaging relating to their obtaining from natural resources and unique morphology, which allow to combine with other polymers, nanoparticles, and additive in order to gain adequate mechanical, thermal, optical, barrier, and antibacterial properties. Although biodegradable polymers and their associated mixtures and composites have been advanced in the world food packaging market in recent decades, the composting and recycling methods for waste management are not applied at industrial scale. For example, after composting, the PLA cannot be separated from the PET, and the PLA's microbial degradation is limited to some strains of microorganisms.

As a viable solution for biodegradable food packaging waste, the developing of innovative edible food packaging was proposed. Edible film and edible coating in food applications respond to the societal challenge concerning climate action, environment and diminishing resources, and raw materials, as well as the increasing of world's population and resources consumption. In accordance with the Food and Agriculture Organization of the United States, each year over 1.3 billion tones of food are wasted, from which horticultural crops (fruits and vegetables) waste represents 45–45% (Singh and Packirisamy 2022). Edible food packaging better solves the problems arising from the preserving of crop in a certain season, with annually balancing of demand and constant maintenance of market prices. Edible films are structures developed like thin layers and then used to coat the food products, as primary edible packaging with the purpose to protect the food, while edible coatings are referred to the dipping of food products into an aqueous solution of biopolymers. By comparison with conventional food packaging systems, edible food packaging systems have the following functions: (i) are an integral part of the food product; (ii) have the role to extend shelf-life; (iii) can be consumed together with packages; and (iv) are biodegradable and environmentally friendly. However, if the food packaging system is not eaten, its degradation occurs fast like the destruction of plastic material. The differences between edible films and edible coatings refer to the thickness and structure. The thickness is greater in the case of edible food films. A difference of about 300 μm in thickness, determined by the density, viscosity, coating time, and surface tension of the biopolymer solutions, was reported (Shahidi and Hossain 2022). The edible food films are self-fixed systems, while those edible food coatings attach to the food product surface (Galus et al. 2020).

The purpose of the present chapter is to investigate the advantages and limitations of the main biopolymers used for fabrication of edible and coating food packaging, their compositions with additives, plasticizers, and bioactive agents, as well as the performance required for use in food packaging applications.

Biopolymers Used for Edible and Food Packaging

Edible food packaging includes films and coatings based on natural and food-grade biopolymers that can be classified into three main groups: polysaccharides, proteins, and lipids (Dhumal and Sarkar 2018; Kumar et al. 2021; Iniguez-Moreno et al. 2021; Vukic et al. 2017) (Fig. 1).

Generally, edible films and coatings achieved by polycarbohydrates are distinguished by excellent gas barrier properties, while those obtained from proteins are considered to have great mechanical properties. However, both polysaccharide- and protein-based edible films display low water vapor barrier properties, which can be enhanced by the introduction of nanoparticles (NPs) such as zinc oxide (ZnO) (Anugrah et al. 2020), lipids, or adding one or more hydrocolloids (proteins and polysaccharides) (Fabra et al. 2014).

Polysaccharides

Among the most naturally occurring biopolymers, a special consideration has been given to polysaccharides, which are found in many components, are easily

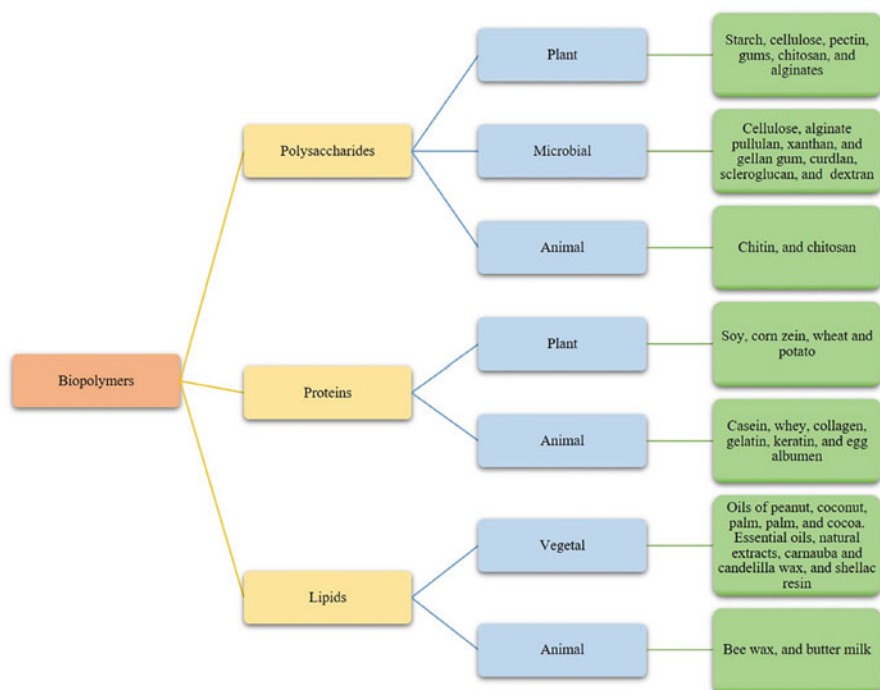


Fig. 1 Classification of biopolymers used for edible food packaging applications (Iniguez-Moreno et al. 2021)

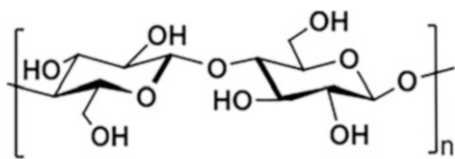
accessible, and have a relatively cheap price. Different natural polysaccharides, for example, alginate, chitosan derivatives, pectin, cellulose, seaweed, and starch, are commonly accepted for fabrication of edible films and coating systems (Marangoni et al. 2021; Poverenov et al. 2014; Rapa and Vasile 2020; Senturk Parreidt et al. 2018; Soares et al. 2018; Kuprina et al. 2021). Polysaccharides, as the main component of biomass, accomplish the environmental considerations (being extracted from sustainable and biodegradable resources) and are potential to be used in edible food packaging, being metabolized by human body together with food. Scientists from the University of Nottingham have employed polycarbohydrates and proteins to design a fully environment-friendly and edible food packaging. Extracted from konjac flour, starch, proteins, or cellulose-sustainable biopolymers, edible food packaging with enhanced storage, safety, and food quality was reported (<https://www.packaging-gateway.com/news/university-nottingham-develop-edible-food-packaging/>).

Wheat bran is an outer skin of grain composed from pericarp, hyaline, aleurone layers, and remaining starchy endosperm cell layers. Commonly, wheat brain comprises almost 13–18% proteins, 12% water, 57% carbohydrates (hemicelluloses, starch, and cellulose), 4% fats, and 1% phenolic acids (Apprich et al. 2014; Luthria et al. 2015). Wheat cell walls contain a great amount of polysaccharide derivatives (for example, arabinoxylan fiber essential for healthy diet) and ferulic acid from phenolic acids acting as a cross-linker that binds to sugar debris. Ferrulic and vanillic acid compounds from wheat bran waste can be used for the production of sustainable bioplastics (Skendi et al. 2020). Agricultural residues are among the most promising next generation of raw materials for obtaining of biopolymers because they not only are naturally biodegradable and produced using renewable resources, but also can be edible, providing food for many people.

The cheapest edible films were prepared by Agceli (2022) from levan biopolymer synthesized by *Pseudomonas mandelii* and powdered ostrich eggshells, in the context of circular economy. The results indicated that the rising amount of ostrich eggshell in powder form led to a decrease in water absorption, water vapor barrier, and strain properties, although the strength and thermal stability of the material increased. Significant increase in the antioxidant activity proven by DPPH radical scavenging assay (83.03%) and antimicrobial effect against *Streptococcus pneumoniae* 6303 and *Pseudomonas aeruginosa* were noted in the case of using edible levan-based films.

Cellulose is a linear homopolysaccharide consisting of D-glucopyranosyl groups connected via β -(1 \rightarrow 4) glycosidic bond (Fig. 2). Nonetheless the use of cellulose to

Fig. 2 Chemical structure of cellulose (Nesic et al. 2020)



achieve food edible films is restricted as a result of its inability to form a solution in aqueous and chemical solvents (Iniguez-Moreno et al. 2021). This disadvantage could be reduced by chemical modification, which really corrects both water solubility and the thermoplastic nature of cellulose. Ethers (for example, carboxymethyl cellulose, methylcellulose, hydroxypropyl cellulose, and hydroxypropyl methyl cellulose) and esters (for example, cellulose acetate) are among the cellulose derivatives found in the manufacturing of edible food packaging.

Chitosan is a marine polysaccharide produced from the deacetylation of chitin. It contains >55% *N*-acetyl-D-glucosamine and D-glucosamine linked via β -(1 \rightarrow 4) glycosidic bonds. Chitosan is extensively employed as a film-forming ingredient for formulation of edible films or coatings with the purpose to protect fruits and vegetables, for example, carrots, pumpkins, avocados, fish, and cheese (El-Sayed et al. 2021; Le et al. 2021; Kang et al. 2022; Park et al. 2022; Poverenov et al. 2014; Soares et al. 2018; Song et al. 2017). A comparison between edible films by casting method based on chitosan and alginate, respectively, both containing *C. tomentosum* seaweed extract (SE) showed that the highest elongation at break was achieved in the case of chitosan formulation, when the SE acted as a plasticizer in film formulation facilitating the increase of polymer molecular mobility (Augusto et al. 2018). The thickness of the designed edible films ranges from 30 to 50 μm . The addition of *C. Tomentosum* to alginate led to the decrease of whiteness index values, a lower degree of film transparency, moisture of 24–28%, and decreased solubility and WVP. In the case of chitosan film, the transparency and solubility increased, moisture was in the range of 9–10%, and a greater flexibility and resistance to mechanical forces were achieved.

Alginate is another marine polysaccharide, which is extracted from many brown algae types (*Phaeophyceae* sp.) and few bacteria, being responsible for the cell walls elasticity and durability. The chemical structure of alginate is composed by guluronic and mannuronic acid units (Fig. 3). Food grade alginic acid sodium salt was

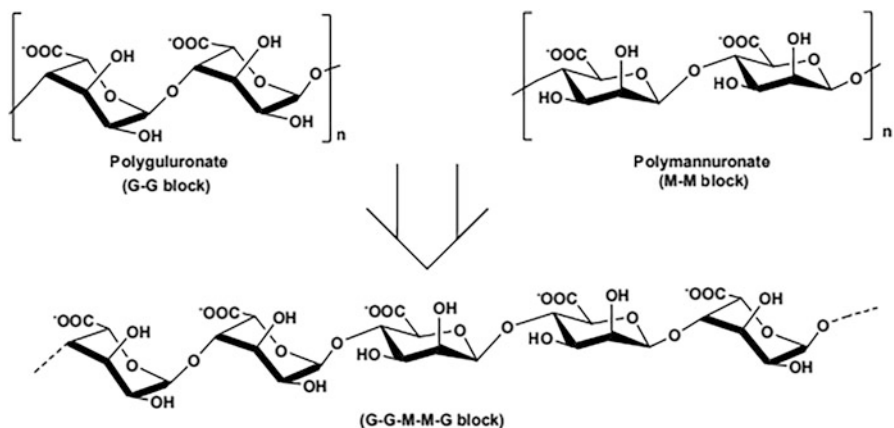


Fig. 3 Chemical structure of alginates (Nesic et al. 2020)

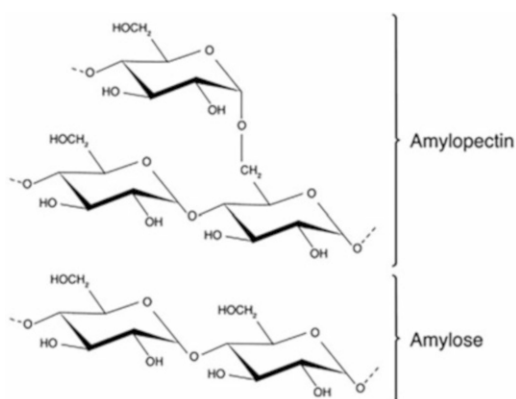
approved by the FDA in the Code of Federal Regulations (CFR) as a GRAS (generally considered safe) and registered as allowed thickener, emulsifier, and gelling and stabilizer food ingredients, being listed under E400–E404 numbers (Lisitsyn et al. 2021). Alginate compositions show acceptable hydrophilic properties and contribute to maintaining moisture, diminishing contraction, and enhancing the sensory attributes of packaged foods. Alginate-based edible layers may diminish the presence of microorganisms, and total losses occur during cooking, delaying the oxidative unpleasant aroma, as well as improving sensory acceptance (Theagarajan et al. 2019). Notwithstanding the capacity to make hard films, alginate films display insufficient ability toward water penetration because of their hydrophilic character. For reducing sensitivity to water vapor of alginate, Marangoni et al. (2021) proposed the combination with hydrolyzed collagen (HC) and nano-SiO₂.

Agar has a high molecular weight assigned to the agarobiose units, providing the support structure of some algal cell walls. Agarophytes are red macroalgae belonging to the Class Rhodophyceae. A composition of agar/alginate/glycerol in a 2/1/1 ratio was cast for obtaining an edible film with adequate physical, optical, and solubility properties for food and food supplement packaging (Puscaselu et al. 2021).

Carrageenans are marine biopolymers, derived from red algae types, which belong to sulfate galactans with linear backbones (Class Rhodophyta). After gelatin and starch, the carrageenans are tertiary very important hydrocolloids of the food industry. The usually commercial carrageenans found are *Eucheuma denticulatum* and *Kappaphycus alvarezii* (Mahmud et al. 2021). Edible coatings for fresh food preservation were obtained by Sousa et al. (2008) by extraction of carrageenan, alginate, and agar from a mixture of dead seaweeds named “Sargaco,” as a cheap alternative to commercial biopolymers. These edible coatings with possible application for fresh fruit preservation showed good water permeability.

Starch is a polymeric carbohydrate consisting of 1, 4-linked D-glucose units. Starch is constituted from two principal forms: amylose and amylopectin, both representing 98–99% from dry weight of starch (Fig. 4). Amylose (25–30%) is

Fig. 4 Chemical structure of starch (Nesic et al. 2020)



α -D glucose joined by α -(1 \rightarrow 4) linear glycosidic chains, which facilitates, in the main, the amorphous region of the starch granule. On the other hand, amylopectin is a branched architecture consisting of α -D-(1 \rightarrow 4) glycosidic bonds. Amylopectin chains subsequently form the peripheral crystalline structure of starch granules (Thakur et al. 2019).

Moisture absorption sensitivity and poor mechanical features are the main drawbacks of starch films for their use as food packaging material as compared with the petroleum polymers. Currently, the scientific studies advise that the introduction of plasticizing components or another active additive could significantly enhance the water vapor barrier properties of starch-based edible films or coatings. Plasticizers are essential ingredients necessary for the fabrication of edible films and coatings because they improve the elongation and strength of polymer matrices (Apprich et al. 2014). Polyols (e.g., glycerol and mannitol) have been documented as the most desirable plasticizer for starch. For making edible films, the starch gelatinization proceeds at a temperature of about 80 °C.

As reserve carbohydrates, galactomannans are encountered in widely albuminous or endospermic seeds. The physicochemical and conformational properties of the galactomannans are mainly described by the ratio between mannose (M) and galactose (G) and the spread of galactose residues in the principal backbone (Cerqueira et al. 2009). Lima et al. (2010) reported that the edible coatings of mangoes and apples performed with a composition of galactomannans, collagen, and glycerol resulted in reductions of 28% O₂ consumption, 11% CO₂ production, and 50%, respectively.

Beneficial properties obtained in the case of food processing by valorization of by-products, like biodegradability, low price, distinct durability, and lighter weight compared with glass have driven to a considerable evolution of edible films (Cuccolini et al. 2013). Thus, one of the most renewable natural resources of interest is pectin, which is the major constituent of plant biomass, ubiquitous in nature. Globally, the market need for pectin is increasing, showing an entire manufacturing capacity of around 45–50 Mton per year, while its demand was around 140–160 Mton per year in 2011. This demonstrates that the industry's interest in this carbohydrate is rising daily. Due to its versatility in nature, pectin and its derivatives are used in various biodegradable packaging materials having the roles in retaining moisture, oil, and aroma barrier, and to diminish the respiration rate and food oxidation (Hoorfar 2014). At acidic pH, low methoxyl (LM) pectin is cross-linked with the help of calcium cations in order to create strong gels. These gels show a greatly durable architecture and provide a barrier against water. LM pectin thin layers are mostly used as edible packaging materials because of their favorable characteristics (Zhang et al. 2014). Other raw materials for obtaining pectin with dry substance above 15% are various marc-based by-products from the agricultural and food industries, such as citrus fruits, apples, sugar beet pulp, and sunflower husks (Belkheiri et al. 2021). The apple mark (with a content of pectin ranging from 15% to 20%) and citrus fruit peel (where the pectin content varies from 30% to 35%), both extracted from the fruit juice industries, are the essential ingredients exploited for obtaining pectin on an industrial scale. Five extraction methods are the

most used in the case of pectin: heating-stirring extraction based on solvent, magnetic induction (EMI), microwave (MAE) and ultrasound assisted extraction (UAE), and enzyme-assisted extraction (Sen et al. 2021).

Proteins-Based Edible Films and Coatings

Developing protein-based functional materials derived from the food industry such as whey protein isolate nanofibers (WPNFs)/glycerol (Gly)/carvacrol (CA)/salted duck egg yolk edible coating (Wang et al. 2020), lysozyme and β -lactoglobulin isolated from eggs and milk (Shen et al. 2021), corn, soy, mung bean, pea, grass pea, whey, and surimi protein sources (Mihalca et al. 2021), which possess especially antioxidant activity, is beneficial for the reduction of environmental pollution and increasing economic value simultaneously. Common proteins used for producing edible food packaging are the following: casein, soy protein, zein, whey protein, collagen, gelatin, egg albumin, and milk protein (Daniloski et al. 2011; de Oliveira et al. 2021; Fu et al. 2021; Gialamas et al. 2010; Kuprina et al. 2021; Lima et al. 2010; Lin et al. 2011; Tao et al. 2018; Talens et al. 2012).

The commercial casein or caseinates adopted within the food sector ranged from 200,000 to about 2,500,000 t (Lisitsyn et al. 2021). Acidic casein is obtained by acidifying of precipitated casein from skimmed milk. Rennet casein is produced by treating of milk with rennet. Caseinates are compounds derived from water-soluble products of milk proteins created by neutralization reaction. Casein is a well-established dairy by-product exploited as an edible coating for numerous foodstuffs, including dairy farm products.

The disadvantage of edible films based on casein is being very sensitive to moisture and water absorption, which highly alters their mechanical and barrier properties. Moreover, casein edible films are often dissolved in water reducing their domain of utilization. However, the use of plasticizer does not improve the mechanical properties of casein-based edible films. One solution consists of crosslinking of polymer chains with glutaraldehyde, genipin, or transglutaminase agents. Sodium-caseinate plasticized with sorbitol with and without *Lactobacillus sakei* cells was cast for the development of a novel bioactive packaging for controlling *Listeria monocytogenes* in foods (Gialamas et al. 2010). The results showed that the incorporation of microorganisms into edible films made of sodium-caseinate did not affect their physicochemical characteristics. Supplementary, it significantly inhibited the *L. monocytogenes* growth in the case of fresh beef (food model system) as compared to the control samples.

Soy is another protein obtained from the solid waste produced by the food industry. Two soy protein types are commercially available: soy protein concentrate (SPC), which contains 65–72% protein, and soy protein isolate (SPI), which has more than 90% protein content (de Oliveira et al. 2021). Due to the amphiphilic character, by denaturation of soy protein, a stable network is formed. De Oliveira et al. (2021) reported that the soy protein can interact with carboxymethylcellulose (CMC) at pH below the isoelectric point (IP) of soy protein (4.5–5.0) to improve the

mechanical strength and oxygen permeability of edible films. The proteins isolated from plants such as corn, wheat, soybean, peanuts, cottonseed, and rice are still suitable and accepted by vegetarian people.

Zein is an amphiphilic protein rich in prolamins authorized by the FDA as a generally recognized safe (GRAS) additive for food applications. Zein is a biodegradable and biocompatible biopolymer, which corresponds to the strategy of agro-industrial by-products management, being deeply studied for the development of edible films (Salevic et al. 2022). Because the films fabricated from zein are brittle, the combination of zein with polysaccharides is preferred (Perez et al. 2021).

Studies performed by Taylor et al. (2005) showed that the kafirin, the prolamins protein extracted from sorghum, is more hydrophobic than zein. The kafirin edible film was obtained by casting method with glacial acetic acid as a solvent, at a temperature of 25 °C, and showed advantages compared to that obtained in the presence of aqueous ethanol, in terms of lower processing temperature and better tensile and water permeability.

Collagen is another extensive protein that is found in all connective tissues including bones, tendons, ligaments, skin, and cartilage, as well as fish waste (Bhuimbar et al. 2019; Mihalca et al. 2021). Collagen is composed of polypeptides and characterized by a triple helix structure, resulting from three cross-linked α amino acid chains consisting of two homologous $\alpha 1$ chains and one $\alpha 2$ chain. Marine sources, mainly fish by-products, such as sea urchin, starfish, and sea cucumbers, have been reported to be rich in collagen (Fu et al. 2021). Collagen films are characterized by an excellent barrier to oxygen, but poor water vapor permeability. Gelatin-plasticizer combinations were explored as potential durable edible films for food (Sancakli et al. 2021). A potential alternative to obtaining edible films with improved performance is to mix the collagen with other polysaccharides. Thus, blending of galactomannan of *A. pavonine*, collagen, and glycerol with the compositions of 0.5%, 1.5%, and 1.5%, respectively, led to obtaining edible films for decreasing the fruit gas transfer rates for mangoes and apples. Therefore, it will play an important role in prolonging the shelf life of postharvest fruits (Lima et al. 2010).

Lipids-Based Edible Films and Coatings

According to a review by Zubair et al. (2021), lipids are biorenewable feedstocks classified as vegetables, fatty acids, plant oils, or waxes, which possess interesting mechanical, barrier, antibacterial, antioxidant, and functional properties to target various applications in the food packaging sector. The use of lipids as matrix, plasticizer, or filler in the edible film or coating is recommended due to their hydrophobic properties and the capability to enhance the mechanical and antioxidant properties.

Lipids do not constitute cohesive films, unlike hydrocolloids. For this consideration, they are used specifically for food coatings or in mixtures with polysaccharides in order to achieve an advanced water barrier property. A new edible and active

film/coating material based on bioactive compounds extracted from *Aloe vera* leaves blended with proteins and polysaccharides was proposed by Maan et al. (2021). This combination of components could be an excellent edible film/coating for perishable food products.

Features of Edible and Coating Food Packaging

General Requirements for Edible Films and Coatings

Edible food packaging is a multifunctional one, characterized by good oxygen barriers, being capable of protecting the quality and expanding the validity of food products sensitive to spoilage. Edible films and coating may impede the oxidation of dried food products during dehydration and enhance the shelf life expansion disclosed by exposure to radiation and can also diminish the flavor, color, or vitamin content in foods that are liquid at room temperature at the time of fabrication and commercialization. They also can be an efficient transporter for antimicrobial and/or antioxidant agents and to manage the transfer of particles from the package toward the food product. In addition, edible food packaging has demonstrated favorable transmission properties, being investigated for food preservation. Edible food packaging may be beneficial for freeze-drying processes, since it can avoid the evaporation of volatile flavors.

Figure 5 illustrates the main steps and laboratory methods needed for fabrication and testing of edible films and edible coatings in food applications.

The first step to fabricate edible food films is concern casting of solution involving the preparing of the biopolymer solution with adequate additives followed by evaporation of the solvent. The prepared solution of biopolymer could be also used for the coating of food products. Natural biopolymers can be used as single, binary, or ternary combinations for creating edible films and coatings. The main functions of biopolymer compositions are the following: (i) to prolong the shelf life of stored food; (ii) to improve the physical characteristics of food products; (iii) to protect the stored food from oxidation, moisture absorption, and microbial growth; (iv) to preserve the high quality of the food product; and (v) to enhance food appearance. Generally, food protection, the physical properties or functionality of edible packaging, cannot be provided with a single biopolymer, and the introduction of plasticizers and other additives to the processing formula is required. Usually, the plasticizers include polyols such as propylene glycol, glycerol, mannitol, sucrose, honey, and fatty acid esters (Singh and Packirisamy 2022). For example, glycerol is a plasticizer used for film-forming solutions in concentrations ranging from 0.5% (w/w) to 2% (w/w) (Perez et al. 2021). The composition of the edible film should be accepted on the basis of the special uses of food, the type of food products, as well as the dominant quality depreciation technique (Talens et al. 2010). In addition, the biopolymers can be combined with other active compounds such as antioxidants, antimicrobial, nanoparticles, essential oils, vitamins, or foodborne pathogenic bacterium to add improved safety, nutritional and sensory features, to edible films

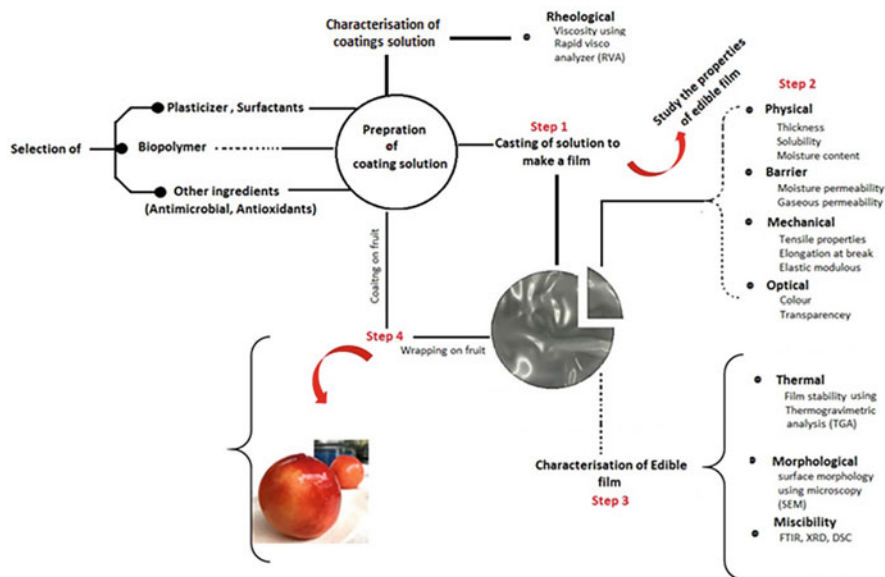


Fig. 5 General steps for the development of edible film and coating for food applications (Thakur et al. 2019). (With permission of Elsevier)

followed by casting of the film (Fabra et al. 2014; Gialamas et al. 2010). Bioactive compounds are added to edible film by the encapsulation process. Depending on its sized dimensions of particles, nanoencapsulation and microencapsulation of bioactive compounds resulted. Thus, the particles sized in the range of 10 to 1000 nm form mini-emulsions of bioactive compounds, while in the case of sized particles of 6 to 800 μm microemulsions result (Chaudhary et al. 2021).

Table 1 shows some bioformulations for edible food packaging prepared on a laboratory scale and tested for fruits and fish.

The following general requirements for ideal edible films or coatings are taken into account: They must not contain harmful, allergenic parts that are not broken down during digestion, and they must allow an acceptable adhesion on the food contact surface, to ensure architectural durability for avoiding mechanical deterioration at the transportation, manipulation, and exposure steps, to keep the migration of additives under regulations in force, to prevent the loss of ingredients that maintain aroma, flavor, and nutritional and organoleptic attributes required for consumer approval without adversely changing these characteristics, and to ensure a partial permeability to keep the internal balance involved in aerobic and anaerobic respiration. All these demands for edible packaging together with the easiness of manufacturing and economical feasibility will delay the senescence and provide the microbiological stability of the food items, maintaining and enhancing their sensory features (taste, appearance, etc.) (Fabra et al. 2014).

Table 1 Some biocompositions for edible food packaging

Composition	Edible food packaging/ technique	Target food	Ref.
Chitosan and chitosan/lauric acid	Coating/vacuum impregnation	Minimally processed pumpkins	Soares et al. (2018)
Caseinates or chitosan	Coating	Partially dehydrated pineapple-cereal system	Talens et al. (2012)
Chitosan-collagen composite	Coating	Fresh red porgy (<i>Pagrus major</i>) file	Liu et al. (2020)
Chitosan/nanosized titanium dioxide particles (CTS-TiO ₂)	Coating	Blueberries	Xing et al. (2021)
Sodium alginate solution	Dipping and vacuum impregnation coating	Fresh-cut cantaloupe melon	Senturk Parreidt et al. (2018)
Sodium alginate (SA)/hydrolyzed collagen (HC)/SiO ₂ nanoparticles	Film by casting		Marangoni et al. (2021)
Alginate/antimicrobial agent active edible coating	Coating/dipping	Fresh-cut Galia-type melons	Poverenov et al. (2014)
1% lactic acid solution (LA)/1% CH containing 64 µg/mL nisin/250 µg/mL ε-polylysine (ε-PL) (LA/CH/ Nisin/ε-PL)	Coating	Fresh-cut carrots	Song et al. (2017)
Soy protein isolate (SPI)/ Carboxymethylcellulose (CMC)/ glycerol	Film/casting		de Oliveira et al. (2021)
Gelatin/starch– butanetetracarboxylic acid dianhydride (BTCAD)– <i>N</i> -hydroxysuccinimide (NHS)	Film/casting	Apple	Tao et al. (2018)
Nanocomposite film based on gelatin prepared from waste fish scale, chitosan, and CaCO ₃ nanoparticles	Film/casting	Longan and banana	Fu et al. (2021)
Galactomannans isolated from <i>Adenanthera pavonina</i> and <i>Caesalpinia pulcherrima</i> seeds, glycerol, and collagen	Coating	Mangoes and apples	Lima et al. (2010)
Zein coatings incorporated with nisin (54.4 AU/cm ²) or nisin/ ethylenediaminetetraacetic acid (EDTA) 568 µg/cm ²	Coating	Fish balls	Lin et al. (2011)
Sodium alginate, chitosan, and protein hydrolysate	Coating	Semifinished fish product	Kuprina et al. (2021)

The specific edible food packaging should be tested from many points of view: mechanical properties, thermal resistance, morphology and other technical features, processability, and consumer acceptance as well as applicability and sustainability versus commercially available plastics used in the food packaging industry. The best mechanical and physicochemical properties of polysaccharides/proteins were investigated by response surface methodology (RSM). In a study conducted by Cortes-Rodriguez et al. (2020), 15 emulsions based on cassava starch (CS) (3.0–3.5%), beeswax (BW) (0–0.5%), and whey protein (WP) (0.5–1.5%) were prepared by casting method. The following properties of compositions were measured: moisture content, swelling capacity, thickness, water vapor barrier, and tensile properties, as well as the color parameters: yellowness index (Y_i), lightness (L^*), saturation (C_{ab}^*), tone (H_{ab}^*), chromaticity (a^*), and chromaticity (b^*). The study revealed that the optimal composition having a possible application in the preservation of fruits contains the following: 3.17% CS, 1.3%, WP (1.30%), and 0.50% BW.

The water solubility and swelling degree of edible food packaging materials influence the rate of the materials' breakdown during consumption and biodegradation (Salevic et al. 2022). Flexibility and mechanical strength are two essential material properties for food packaging associated with the capability of edible films to support the external force and keep completeness and barrier properties. The optical properties of edible films are essential for commercial appearance, leading to consumer acceptance. The color, firmness, reducing of sugar content, and weight loss of coated food are properties also evaluated during the storage period to predict prolonging shelf life of the tested food. The release of bioactive components from edible films involves the control of dissolution, swelling, and diffusion of bioactive agents. The wrapping/coating process used depends on the quality of food item's surface and the goal of coating layer. Coating development commences with the diffusion of the components on the food surface, being accompanied by the adherence of coating formulation with the food surface. The initial concentration of coating solution should be correlated with the coating film thickness as the firmness properties and appearance of the coated fruits should not be disturbed. It is accepted that the protective layer for the coating of food should be uniform and smooth.

Common techniques used to prepare edible films are the following: wet technique or solvent casting, dry techniques such as compression molding or extrusion as well as electrospinning. The wet process involves dispersion of biopolymers into solvents, and spreading of the obtained solution on the adequate substrate, then the evaporation of the solvent takes place. Encapsulation is a new technique for combining edible films with bioactive compounds, flavor compounds, and probiotics that provide controlled release and health benefits. Dry process, compression molding, or extrusion uses the high temperature above the glass transition temperatures of biopolymers, pressure, and adequate processing additives. Edible coating of food can be achieved by three methods: spraying, dipping vacuum impregnation, and soaking and electrospinning. Table 2 shows the main advantages and disadvantages of each edible food packaging method.

Chitosan-based edible coatings achieved on minimally processed pumpkins by soaking, and vacuum impregnation techniques reveal that the soaking is considered

Table 2 Advantages versus disadvantages of methods for preparing edible films and coatings for food applications

Technique	Advantage	Disadvantage	Reference
Edible films			
Solvent casting	Continuous and compact films were obtained A simple method, which does not require specific equipment	Compatibility of solvents with biopolymers Low availability at the industrial scale	Salevic et al. (2022); Singh and Packirisamy (2022); Taylor et al. (2005)
Electrospinning	The incorporation of bioactive compound in biopolymeric matrix extending the functionality of film	Low availability at the industrial level Compatibility of solvents with biopolymers	Salevic et al. (2022); Singh and Packirisamy (2022)
Compression molding and extrusion	Large-scale production	May affect the film properties (thermal degradation)	Chuaynukul et al. (2018)
Coating/antimicrobial coatings			
Dipping	Enhance the quality of fresh-cut fruits Increase the structural integrity of food Decrease of microbial spoilage Increase storage period of fruits Prevent the loss of volatile compounds Good esthetic appearance of fruits	Good adhesion is not achieved if food products' surface is hydrophilic Nonuniform thick coating Multilayer coatings	Poverenov et al. (2014)
Spraying	Small amount of coating solution is required due to the high pressure used Uniform thickness	The viscosity of coating solution should be controlled	Anugrah et al. (2020)
Electrospinning	Protect the bioactive compound by nanoencapsulation		
Vacuum impregnation	Thicker film	Greater component incorporation in the food product	Soares et al. (2018)
Soaking	Lower numbers of psychrotrophic microorganisms and coliforms Smaller change in the food product		

the best technique since it did not lead to changes in the properties of the food, as compared with vacuum impregnation, when higher modifications in pH, acidity, color, and firmness were produced (Soares et al. 2018). In a study performed by

Senturk Parreidt et al. (2018) about the comparison between dipping and vacuum impregnation techniques in the case of coating of melon pieces with sodium alginate solution, many coating parameters such as dipping time, draining time, time length of the vacuum period, vacuum pressure, and atmospheric restoration time were analyzed to obtain data about the percentage of weight gain, color, and texture of coated sample. It was found that although both methods improved the firmness of the tested food, the vacuum impregnation led to better mechanical and structural properties.

Singh and Packirisamy (2022) performed both dip-coating and electrospinning methods for coating of perishable banana and apples with a composition based on silk fibroin (SF)/poly(vinyl alcohol) (PVA) solution carrying nanocurcumin as bioactive agent and honey as natural moisturizer, as a cost-effective method. Dip-coating of the model foods was achieved by directly immersing them three times in the SF/PVA aqueous composite at 5-minute intervals for 30–40 s; next the coated food was kept for drying at room conditions, while in the case of the edible nanofiber coating, the optimized parameters were fixed at a volume of 0.5 mL which passes per hour, and the voltage of 20 kV. In both cases, the coated food models exhibited a prolonged shelf life as compared with uncoated foods.

Performance of Edible Food Packaging

The interest in active edible film/coating is increasing due to the extended shelf life of food by using natural antimicrobial components as substitutes for artificial supplements. Edible food packaging also has the role of carriers of bioactive substances and promotes the health of consumers. Some examples of bioactive molecules used for edible food packaging are the following: antimicrobial and antioxidant compounds (ϵ -polylysine (ϵ -PL), resveratrol), sage extract, clove bud oil, ZnO NPs, microcrystalline cellulose, honey, nanocurcumin, and pomegranate peel extract (PPE) (Bhuimbar et al. 2019; El-Sayed et al. 2021; Garcia et al. 2022; Kang et al. 2022; Le et al. 2021; Leena et al. 2020; Park et al. 2022; Salevic et al. 2022; Singh and Packirisamy 2022), probiotics (*Bifidobacterium lactis*, *Lactobacillus acidophilus*, and *Lactobacillus casei*) (El-Sayed et al. 2021; Pop et al. 2020), antienzymatic agents, polyunsaturated fatty acids, and different nutraceutical types.

Medicinal and aromatic plants achieved a huge concern in the food sector due to their active ingredients that are frequently discovered in the extracts/essential oil fractions. It is well proven that most of them show a large spectrum of activity for killing and inhibition of microorganisms leading to deterioration of food (Gavril et al. 2019; Gutierrez et al. 2008; Vasile et al. 2019).

Table 3 shows few examples of compositions of biopolymers together with bioactive components for obtaining of edible food packaging and the most significant properties reported for them.

Garcia et al. (2022) prepared four different biopolymer coating solutions referred as: zein solution (ZS), zein nanoparticle suspension (ZNP), and ϵ -polylysine solution (PL), and zein- ϵ -polylysine nanoparticle suspension (ZPL), together with a test

Table 3 Active edible food packaging compositions and related properties

Composition	Food packaging and method	Properties	References
25% w/v of zein in 80% aqueous ethanol/ glycerol, 2.5% w/v/ of resveratrol (containing 0, 2, 5, and 10% w/w of polymer)	Electrospinning at following optimized conditions: 8 cm distance from the spinneret tip, voltage of 14 kV, and a constant flow rate of 0.5 mL/h	DPPH scavenging abilities of resveratrol-loaded zein nanofibers differed from about 66.2 to 81.1% keeping the antioxidant capacity of resveratrol The fastest release of resveratrol up to 39% was registered at 2 h immersion in PBS Nanocoating of apple thin slices revealed an acceptable controlled moisture loss and maintained their color during the 6-h examination period	Leena et al. (2020)
Zein and ϵ -PL nanoparticles (ZPL), zein nanoparticles (ZNP), ϵ -PL solution (PL), and zein solution (ZS) coating films together with control (C)	Dip-coating	Up to 36 days of study, the ZPL coating showed the inhibition of fungal ZPL and ZNP coatings exhibited good physical appearance and texture of avocados as compared with control	Garcia et al. (2022)
Zein (25% w/w in aqueous ethanol solution (80% v/v))/ sage extract (SG) (10, 20, and 30% w/w)	Solvent casting mono-axial electrospinning	The solubility and swelling degree in water increased with the content of sage extract Similar DPPH• and ABTS•+ radical scavenging abilities for film and mats Inhibition the growth of <i>S. aureus</i> and <i>S. enterica</i> serovar Typhimurium bacteria	Salevic et al. (2022)
Edible films containing red cabbage (RC) and clove bud oil (CBO) filled with chitosan or carrageenan capsules	Encapsulation using the ionic gelation method	High potential for use in fresh fish preservation	Park et al. (2022)
Solutions of 1% chitosan and 0.03 g ZnO nanoparticle, respectively, were prepared into 1% acetic acid solution	Coating	Extend the shelf life of wild-simulated Korean ginseng root The growth of <i>B. cereus</i> and <i>E. coli</i> was inhibited	Kang et al. (2022)

(continued)

Table 3 (continued)

Composition	Food packaging and method	Properties	References
1% (wt/v) chitosan dissolved in 0.25% acetic acid solution and 10% (w/v) gum Arabic dissolved in distilled water with 0.1–1% (wt/wt) zinc oxide (ZnO) nanoparticles film-forming edible coating	Coating of avocados	0.3% w/w ZnO NPs incorporated into polysaccharide formulation led to the optimal protective performance of avocado's surface after 7 d (negligible weight loss, improvement in lightness, appearance and firmness, and reducing sugar till 18.5 mg/g)	Le et al. (2021)
Chitosan (CH)/sodium alginate (SA)/carboxymethyl cellulose (CMC)/microcrystalline cellulose (AMCC)/probiotic strains (<i>Lactobacillus acidophilus</i> , <i>Bifidobacterium lactis</i> , and <i>Lactobacillus casei</i>); the used plasticizer was 10% glycerol, while a crosslinking agent was 2% citric acid (CA)	Edible coating for UF soft cheese	AMCC had the role to decrease the WVTR, increase mechanical properties as well as enhance the antimicrobial activity against <i>Staphylococcus aureus</i> , <i>Listeria monocytogenes</i> , <i>Salmonella typhimurium</i> , <i>Bacillus cereus</i> , <i>E. coli</i> , <i>Aspergillus flavus</i> , and <i>A. niger</i> The overall acceptability of cheese was demonstrated by sensorial analysis, after keeping for 45 d at temperature of 7 °C	El-Sayed et al. (2021)
Silk fibroin (SF)/3% PVA/nanocurcumin (250 µg/mL/5% honey composite solution	Dip-coating electrospinning	Hydrophilicity moderated Antibacterial activity against <i>S. aureus</i> and <i>E. coli</i> bacteria and antioxidant activity due to the nanocurcumin Increased shelf life of banana and apple coated with electrospun nanofibers with 4 d and about a month, respectively Dip-coated apple slices showed a better morphology, texture,	Singh and Packirisamy (2022)

(continued)

Table 3 (continued)

Composition	Food packaging and method	Properties	References
		and color as compared with uncoated slices	
3.5% (w/v) collagen/1% (w/v) chitosan film/5% pomegranate peel extract (PPE)/0.75 mL g ⁻¹ glycerol film	Edible film by solution casting	Antimicrobial activity against <i>Bacillus saprophyticus</i> , <i>Bacillus subtilis</i> , <i>Salmonella typhi</i> , and <i>E. coli</i>	Bhuimbar et al. (2019)
Cactus mucilage (CM), agar(A)/glycerol active food packaging	Edible film by solution casting	Good mechanical and thermal properties and moderate antioxidant characteristics (Young's modulus ≥ 135 MPa; tensile strength ≥ 5.3 MPa; transmittance in UV range $\leq 40\%$; thermal stability = 140 °C; water vapor transmission rate (WVTR) ≤ 10.6 g h ⁻¹ m ⁻² ; DPPH radical scavenging assay $\geq 19\%$; and ferric reducing antioxidant power ≥ 1.3 mg AAE per g of film)	Makhloufi et al. (2022)
Soluble soybean polysaccharide (SSPS)/ Zataria multiflora Boiss (ZEO) or Mentha pulegium (MEO) essential oils films	Solution casting	Improved water solubility and water vapor barrier properties Compatibility of essential oil with SSPS proved by evidence of single Tg (DMTA test)	Salarbashi et al. (2013)

witness (C) in order to evaluate their influence concerning the extent of shelf life for 220 Hass avocados. All fruits were coated in one of the five coating solutions. The coated fruits were rapidly removed from the coating solutions followed by drying in the air 1 h. The data showed that the coated avocados with ZPL and ZNP compositions led to less weight loss, less breathing, more firmness, and less color change. Up to 36 day of study, at room temperature, the ZPL and ZNP coatings led to no changes in the initial physical appearance and texture of avocados as compared with control. The antimicrobial ϵ -PL solution added to zein (ZPL coating) favored the inhibition of fungal incidence.

A different preservation approach for cut carrots to keep them fresh was developed by Song et al. (2017), who investigated the association of nisin/ ϵ -polylysine (ϵ -PL)/chitosan (CH) as coating solution. After the storing of coated cut carrots at 4 °C for 9-day storage, the modifications in sensory qualities, physicochemical indicators, respiratory rate, microbiological growth, and white blushing were recorded. The authors demonstrated that the lactic acid (LA)/CH/Nisin/ ϵ -PL coating solution significantly ($p < 0.05$) obstructed the respiration rate, decreased the ascorbic acid and expansion of microorganism (*S. aureus* and *Pseudomonas spp.*, mold and yeast, total coliforms count, and total viable count), and raised total phenol content and phenylalanine ammoniolyse (PAL) properties related to the control. The acceptance of consumers carried out by a sensorial panel was enhanced, proved by the strong inhibition of the white blush of fresh-cut carrots. Also, the LA/CH/Nisin/ ϵ -PL coating solution significantly ($p < 0.05$) decreased the lignin formation in fresh-cut carrots, as well as Dc4CL and DcC4H gene expression. Thus, it is supposed that the synergetic effect produced by small intercellular peptides (nisin and ϵ -PL) should have an essential role in plant growth and development.

Leena et al. (2020) developed bioactive edible films based on zein 10% glycerol and resveratrol. The plasticizing effect of glycerin was evidenced by reducing the electrical conductivity and apparent viscosity and increasing the surface tension of the zein solution, which resulted in increased fiber diameter. The high efficiency of $97 \pm 2\%$ nanoencapsulation was permitted by incorporation of only 2% resveratrol into zein composition, while the high content of antioxidant agent could not be totally encapsulated in the zein matrix wall.

Four edible coatings based on caseinates or chitosan were performed for investigation of the effectiveness of coating of partially dehydrated pineapple-cereal system (Talens et al. 2012). After 6 months of analysis of coated systems at 20 °C, the study evidenced that caseinate-based coating by vacuum impregnation technique prolonged the shelf life of the food product with respect to coating made with chitosan. Covering the fruit with films based on caseinate applying by vacuum impregnation technique and followed by the drying of the product highly extends the service life of the pineapple-cereal combination in respect to other coating methods. For the 6-month storage period, the coatings based on chitosan were not capable to prevent the cereals from gaining the demanding moisture content.

The study performed by Tao et al. (2018) showed that the gelatin film was cross-linked with starch/butanetetracarboxylic acid (BTCAD)/dianhydride-*N*-hydroxysuccinimide (NHS) (SBN), a novel macromolecular cross-linker, with different ratios of SBN to gelatin (0%, 5%, 10%, 15%, 20%, 25%, and 30%) to form an innovative edible film by casting for coating of peeled apples. It was shown that the weight loss of the coated peeled apples during 25 days was about 17% ($p < 0.05$) compared with that of native film, which recorded a weight loss of 73%.

Poverenov et al. (2014) coated the freshly cut melons with various solutions of alginate in concentration of 0.5%–6% (w/v), 2% chitosan, 1.5% alginate-2% chitosan matrix, and 1.5% alginate-0.5% citral. The authors established that the combination between alginate and chitosan favored a good adhesion of coating and evident antimicrobial protection for fresh cut melon. The coating time of fresh cut melon was 2 min into alginate solution/alginate/antimicrobial solution and,

additionally, 2 min in an aqueous solution 5% of CaCl_2 , necessary for cross-linking of alginate COOH groups.

A compatibility between sage extract and zein matrix was reported by Salevic et al. (2022), who prepared edible films by casting and electrospinning methods. The antioxidant activity of both zein/sage extract casting films and electrospun mats determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical and 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($\text{ABTS}^{+\bullet}$) radical cation scavenging capacity assessments as well as antimicrobial activity against *S. aureus* and *Salmonella enterica* subsp. serovar Typhimurium are illustrated in Fig. 6a–d.

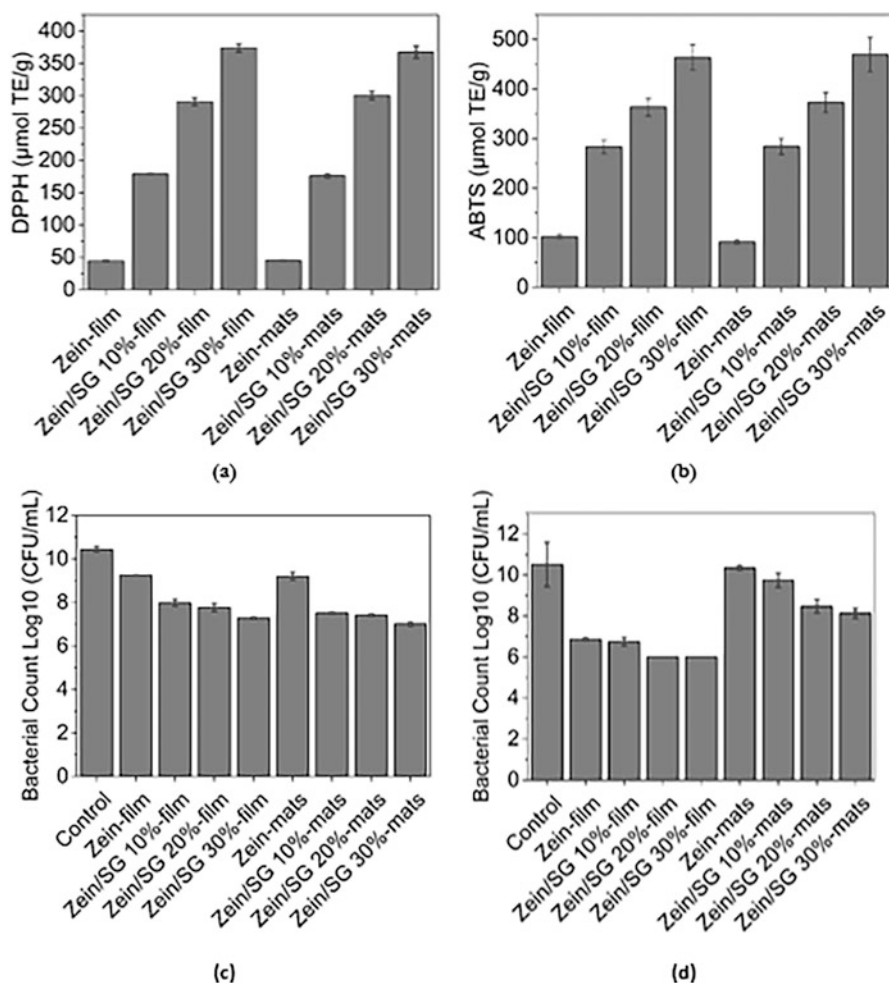


Fig. 6 Bioactive properties of the zein/sage extract obtained by solvent casting versus electrospinning method: (a) DPPH• scavenging ability; (b) $\text{ABTS}^{+\bullet}$ radical cation; (c) *S. aureus*; and (d) *Salmonella enterica* subsp. serovar Typhimurium. (Adapted from Salevic et al. 2022)

In Fig. 6a, b, we see an enhancement in the capacity to scavenge both radicals by the sage extract incorporation, due to the phenolic compounds from its composition. No difference is observed in the antioxidant activity of edible films prepared by those two methods. Initial antioxidant activity is due to the amino acid's residues and short peptides, and xanthophyll pigments from the chemical structure of zein protein, acting as free radical scavengers. The growth of tested foodborne pathogens is reduced due to the inherent antimicrobial activity of sage extract. Another observation from the antimicrobial test refers to the materials with the same compositions, prepared by different methods and their influence on the foodborne pathogens. Thus, the inhibition on growth of *S. aureus* was more evident in the case of electrospun mats than in the case of casting films (Fig. 6c, d). The films prepared by solvent-casting method highly inhibited the growth of *Salmonella enterica* subsp. serovar Typhimurium compared with the behavior of electrospun mats. These results can be explained by the difference in surface antibacterial efficiencies (Salevic et al. 2022).

Clove bud oil (CBO, *Syzygium aromaticum* L.) was used as a natural antimicrobial material for preparing edible chitosan-based films containing red cabbage (RC) as spoilage indicator and antimicrobial agents for fish preservation (Park et al. 2022).

The improvement of edible films was proposed by El-Sayed et al. (2021), who incorporated *Bifidobacterium lactis*, *Lactobacillus acidophilus*, and *Lactobacillus casei* probiotic strains into different polysaccharides and antimicrobial microcrystalline cellulose (AMCC) for coating of UF soft cheese surfaces. The coating of cheese cubes was performed by suspending of cheese cubes into control (containing probiotic strains), and different solutions of biopolymers containing 1% ACMC and 3% probiotic strains for 2 h at ambient temperature.

Natamycin and nisin are antimicrobials approved as food additives for edible food packaging. A composition based on zein, tapioca starch, glycerol, natamycin, and nisin edible film was tested by casting method for producing edible films with a thickness of 0.162 ± 0.002 mm and improved mechanical properties in terms of the decreased elongation at break, barrier property, and film dissolving in water without reducing the antimicrobial activity of film (Perez et al. 2021).

Lin et al. (2011) demonstrated the best antimicrobial activity of EDTA ($568 \mu\text{g}/\text{cm}^2$ of zein film) incorporated into nisin/zein coating against the growth of *E. coli*, *E. aerogenes*, and *C. freundii* for coated fish balls as a commercial product. In 15 days of experiment under cold storage of 4°C , the microbial count decreased from 3.19 ± 0.03 log CFU/g for uncoated fish ball to less than 1 log unit for coated food products, while the control recorded a microbial count of 6.08 ± 0.23 log CFU/g.

Extending the shelf life of avocado was achieved by means of an edible coating based on chitosan, gum Arabic, and ZnO NPs (Le et al. 2021). It was shown that the increasing of ZnO content to 0.5% led to the coated avocado with rough surfaces, due to the agglomeration of ZnO NPs. The preservation effect of the coating layer on the avocado's surface was evaluated by the luminosity parameter on the pericarp of the avocados and by the internal appearance of the avocados cut in half (Fig. 7). After 7 days of coating with edible formulation, it is observed that the avocado had

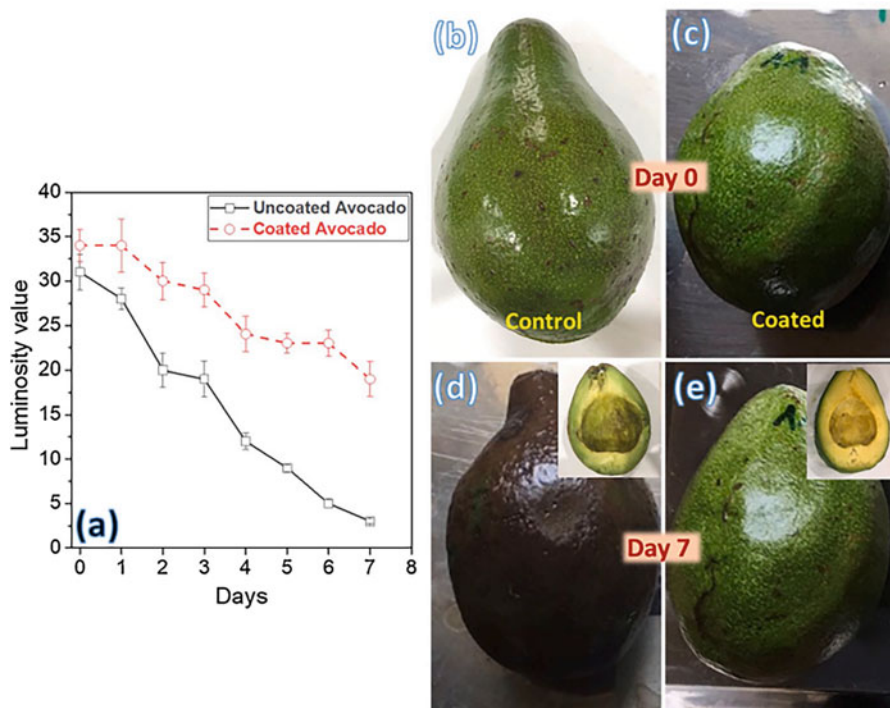


Fig. 7 (a) Luminosity parameter of the avocados treated with chitosan/gum Arabic/ZnO NPs edible coating during the 7 days of storage, appearances of uncoated (b, d) and coated (c, e) avocados. (Permission from Elsevier) (Le et al. 2021)

an improved surface (Fig. 7e) as compared with control (Fig. 7(d)), due to the glossy texture of the edible coating. Also, the pericarp of coated avocados remained unchanged in contrast to that of the uncoated avocado, for which the color was gray. Another advantage of coating is that during the ripening process, the mass loss of avocados is not significant. These are attributed to the antimicrobial activity of ZnO NPs.

Another antimicrobial coating was prepared from chitosan and nano-TiO₂ by Xing et al. (2021) to preserve the quality of coated blueberries at 0 °C. The authors reported that after 32 days of storage at 0 °C, the treated blueberry showed a high firmness, a rapid increase of sugar, acids, and vitamins, from day 4 to day 16 of storage, indicating the postharvest quality and maturity of the fruit, an increase in the ascorbic acid amount in the case of control (11.9 mg/100 g), chitosan (14.57 mg/100 g), and CTS-TiO₂ (13.45 mg/100 g) composite coating, respectively. Further, the enhancing of the anthocyanin, total polyphenol, flavonoid content, and the antioxidant ability of the edible CTS-TiO₂ composite for coated blueberry fruits have improved.

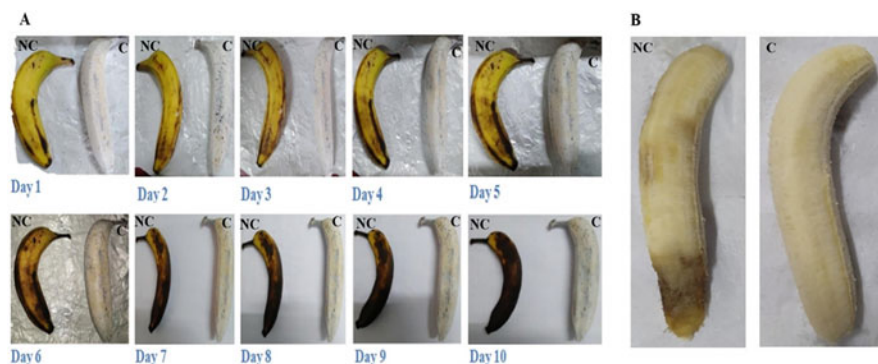


Fig. 8 Edible nanofibers coating of banana (coded C) with SF/PVA composite solution (an electrospinning time of 150 min was necessary to change the position of the banana to favor the adequate coating of all parts) and uncoated banana (coded NC) and their appearance up to 10 days (a); and Banana without peel, on the 6th day (b). (Permission from Elsevier) (Singh and Packirisamy 2022)

An edible coating composition approved by the Food and Drug Administration (FDA), based on silk fibroin, PVA, honey, and curcumin, was processed by electrospinning and dip-coating methods for protection of perishable bananas (Singh and Packirisamy 2022). It was found that the banana coated (C) with edible SF/PVA solution loaded with active and antimicrobial agents enhanced its texture and quality for 8–9 days as compared with uncoated banana (NC) when the good conditions were maintained for only 3–4 days (Fig. 8). In addition, nano-coatings provided extra nutrition to coated food products.

Hydrophilic protein-polysaccharide-based nanocomposite edible film was prepared by Fu et al. (2021) by valorization of fish gelatin and proved to have multifunctional characteristics, such as UV absorption, antimicrobial, oxygen screening, and mechanical and nontoxic properties. In addition, the shelf life of longan and banana was prolonged by 3 days and 5 days, respectively, when compared with the control groups.

Bhumbar et al. (2019) found that the adding of 1% pomegranate peel extract (PPE) to collagen extracted from fish waste/chitosan film did not modify its solubility, while the increased amount of PPE to 5% has the reduced solubility effect declining solubility immensely (36.3% and 18.1%, respectively). Furthermore, the structural integrity of all films was maintained after 24 h and the antimicrobial activity of films increased with the content of PPE.

New edible food-packaging materials were created by Susmitha et al. (2021) with the purpose to valorize the mango and pineapple by-products. Thus, film-forming solutions set up on corn starch/gelatin (CSG) with various concentrations of mango puree with peel (MPP), mango puree (MP), and pineapple pomace (PP), employing glycerol and sorbitol as plasticizers, were mixed and cast for obtaining the edible

films with thickness ranging from 0.22 to 0.30 mm and thermal stability up to 200 °C. The tests showed that the moisture content of edible films increased, tensile strength and elongation at break decreased, maybe due to the presence of free sugars such as glucose, fructose, and sucrose from mangoes, which acted as plasticizers. Adding of puree and peel of mango and pineapple pomace to polysaccharide matrices decreased the water solubility and extended the shelf life of food due to the natural antioxidants from MP, MPP, and PP components.

Recently, an antimicrobial edible food film was produced by Zhang et al. (2022) by casting from edible insects such as grasshopper protein, 30% soy protein isolate (SPI), and cinnamaldehyde (CIN) as an antimicrobial agent. In the presence of 10% xylose as a crosslinking agent, the tensile strength reached 3.4 MPa, elongation at break was of 38%, and antimicrobial activity toward *E. coli* and *S. aureus* increased with the decrease in the water vapor permeability.

Regulatory and Risk Concerns for Edible Films and Coatings

The regulations for edible films and coatings stipulate that all packaging components should be generally recognized as safe (GRAS) in order to keep the product safe and eating quality. In the case when the producer should prove the safety of edible packaging, even the biopolymer-based materials are not GRAS, a GRAS Affirmation Petition to the FDA should be filed or to market without FDA concurrence (self-determination) (Galus et al. 2020).

Edible food packaging must fulfill the Regulation (EC) No 1935/2004, which stipulates the general requirements for all food in contact with packaging, as well as the Regulation (EC) No 2023/2006 which lays down the rules on good manufacturing practices for materials and articles intended to come into contact with food.

In addition, each antioxidant, antimicrobial, food additive, and nanoparticle incorporated into biopolymer formulations with the purpose of improving the performance of edible films or coatings should be identified on food packaging conforming to the European Union regulations, including their substance name or E-number.

Usually, the use of whey, casein, soybeans, fish, peanuts, nuts, and gluten for food coating generates allergens. The manufacturers are obliged to state on the label, the quantity of allergens with the appropriate warning about the allergenic ingredient (Jan and Naig 2021).

Essential oils used as antioxidant and antimicrobial agents for ready-to-eat fruits are analyzed and recorded by the European Commission as well as by the United States as GRAS; however, they may induce allergic reactions. The low concentration of essential oil does not have the expected effect, while the high content leads to undesired organoleptic impacts inducing oral toxicity.

Conclusion and Future Trends

Innovations in the food packaging sector have triggered development of edible films and coating to improve the quality of food, in a sustainable way, and with purpose to reduce environmental impacts.

Biopolymers based on polysaccharides, proteins, and lipids are promising materials used to expand the future of edible food packaging. To obtain optimal properties related to moisture, mechanical strength, and enhancing the shelf life of packaged food, special bioactive compounds are added to biopolymeric matrices, which increases the cost of final products. The composition of edible food packaging must comply with the food regulation regulations of each country (Iniguez-Moreno et al. 2021). However, special attention should be drawn to the solubility of these additives in biopolymers in order to not exceed the specific limits imposed by Regulation 1935/2004/EC (Mahmud et al. 2021).

It is still necessary to expand the fabrication of edible films from laboratory scale when the compositions are poured into Petry plates to scale up and to test the innovative formulations in contact with different foods (fruit, vegetable, fish, or meat).

The trends in edible film and coating are to find new and potential resources of raw materials for biomaterials, like eggshells, as well as to develop edible probiotic coating and nanocoatings. Edible insects are a novel source for edible food packaging with many health benefits (Jeong and Shin 2020).

Nonetheless, to check out if the final quality and real effectiveness of the edible films or coatings are able to safeguard packaged food, a sensory evaluation should be carried out. The aim is to demonstrate that the edible food packaging has not changed the taste and flavor of the final food product.

Cross-References

- ▶ Amylose–Amylopectin Ratio
- ▶ Biopolymers from Marine Prokaryotes
- ▶ Material Applications of Gelatin
- ▶ Packaging Applications of Biodegradable Nanocellulose Composites
- ▶ Soy Protein Biopolymer

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Peptide-Based Biopolymers in Biomedicine and Biotechnology 39

Rini Thresia Varghese, Cintil Jose Chirayil, and Sabu Thomas

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Abstract

The emergence of peptide- and polypeptide-based materials in the field of biomedicine and biotechnology is gaining importance due to its unique physical, chemical, and biological properties like biocompatibility, tunability, ease of synthesis and removal from body, and lack of toxicity. These biocompatible materials are the most suitable for biomedical applications in vivo. The clear

R. T. Varghese (✉) · S. Thomas
School of Energy Materials, Mahatma Gandhi University, Kottayam, India
e-mail: sabuthomas@mgu.ac.in

C. J. Chirayil
Department of Chemistry, Newman College, Thodupuzha, India

understanding of the protein-structure function and their self-assembling mechanism can pave way to obtaining molecular models suitable for different biomedical applications. This chapter aims to focus on the basic properties, synthesis techniques, design principles, and biomedical applications of peptide-based biopolymers.

Keywords

Peptides · Biopolymers · Peptide-based polymers · Biomedicine

Introduction

Polymers represent a long chain entity of different repeating monomers. They can be classified into synthetic polymers and biopolymers. Biopolymers are obtained from natural resources or living organisms like animals, plants, bacteria, and algae. These can be further divided into polypeptides, polysaccharides, and polynucleotides. One of the major areas of biopolymers includes peptide-based polymers which are derived from basic building blocks, amino acids. Polypeptides represent a general class of polymers comprised of repeating chain of different amino acids. However, there are two types of polypeptides, synthetic and natural occurring. Peptides are small molecules rather smaller than proteins consisting of about 2–50 amino acids. The short chains of amino acids are linked together by a chemical bond called peptide bonds. The chains which consist of less than 20 amino acids are called oligopeptides which can include dipeptide, tripeptide, tetrapeptide, etc. These are essential for several vital functions in the body. There are different peptides that are of medical use since they possess antioxidant, antimicrobial, and antithrombotic properties. There are more than 60 peptide-based drugs that are approved and available in market in the USA (Lau and Dunn 2018).

Synthetic peptide-based polymers include engineered peptide-based polymers with advanced properties that can be utilized for different applications. There have been interesting reports on the use of these engineered synthetic biomaterials in the field of biomedicine and biotechnology. Bioinspired materials are more capable for obtaining biomaterials that are capable of obtaining and fulfilling the various needs of the human body. Several novel functional materials comprising of polypeptides with polymers have been reported to have applications in biomedicine and biotechnology especially for the fabrication of scaffolds, drug delivery carriers, biosensing, etc. A clear understanding of the protein–structure function is crucial for developing new repetitive polypeptide-based materials. In contrast to natural biopolymers, engineered polypeptides can be controlled with respect to chain length, mono-dispersity, and stereochemistry (Varma and Gopi 2021).

This chapter provides an insight into the design and principles of peptide-based biopolymers, their properties and characteristics, and their applications in the field of biomedicine and biotechnology.

Peptide-Based Biopolymers

Structure: Almost all the physical, chemical, and biological properties of peptide-based biopolymers depend on structure functionalities be it the primary amino acid sequence, the secondary and 3D tertiary structure. The various interactions between different chain moieties of the sequence result in different characteristic properties of the peptide and polymers.

Properties: Peptide-based biomaterials are mostly soft and wet enabling them to be used in aqueous environment. Thus, they are a blessing for applications where an aqueous environment is required since they can come in contact with water surfaces. In this aspect, they are completely different from traditional materials like metals and ceramics use in bioscience which are hard and not suitable for use in aqueous environment.

The properties of peptides mainly depend on their basic building block, amino acids. However, the number of naturally occurring amino acids is less which is an advantage compared to synthetic polymers. These biocompatible peptide-based materials possess the most important advantage, which is they degrade easily and harmlessly within the body through the usual metabolic process, and they can be widely employed for drug delivery vehicles and tissue-engineering applications. All the physical, structural, and rheological properties need to be carefully studied to evaluate the functionality of these biomaterials. Biophysical properties like molecular weight can be determined by MS or MALDI-MS, and protein concentration can be noted spectrophotometrically or using colorimetry. The clear idea of hydrodynamic radius and protein folding are crucial for applications in protein purification, and drug delivery.

Self-assembly and other ways of directed assembly of various peptides are methods to obtain different functional biomaterials for therapeutic use. Ranging from very small peptides, these can self-assemble to different nano-morphologies like vesicles, micelles, and nanotubes that enhances their stability and makes them suitable for encapsulation of other moieties. Also in terms of biocompatibility, immunogenicity, and biodegradability, the self-assembling peptides typically exhibit favorable features that can result in nontoxic waste products (Ghosh and Chmielewski 2004).

Different Types of Polypeptide Proteins

Compared to synthetic polymers, biomaterials derived from recombinant proteins like silk-like polypeptides (SLPs), elastin-like polypeptides (ELPs), collagen-like polymers, extended recombinant polypeptide (XTEN) polymers (Saxena et al. 2005), and archetypal peptides including leucine zippers are suitable alternatives for use in biomedical applications. These are the protein polymers, comprised of natural or de novo engineered repeated amino acid sequences obtained by the recombinant biosynthesis or via genetic engineering. The main advantage is the tunability of the molecular weight, sequence, length, and monodispersity of the

resultant material. Also the most striking feature is the biodegradability and biocompatibility of these protein polymers owing to the natural polypeptide chains present. Hence the metabolic products comprise of only small peptides and amino acids (Er et al. 2000).

Silk-like polypeptides: Spiders of the order Arachnida, which are arthropods, create silk, a natural protein, and it is also reported that silkworm caterpillars of the order Lepidoptera have exceptional material properties. For instance, spider silk is found to be a light-weight, elastic material with good impact resistance. They are water insoluble and a fibrous protein which consists of sericin and silk fibroin (SF) which are two main proteins. Sericin is a hydrophilic and amorphous protein which is composed of 18 amino acids in a nonrepetitive arrangement. However, sericin may cause immunogenic reactions hence they are separated from SF prior to use in biomedical applications. SF is a high molecular weight, amphiphilic block copolymer protein complex held together by a single di-sulfide bond. The hydrophilic portion of SF is made up of nonrepeated sequences, whereas the hydrophobic portion is made up of a highly repetitive dipeptide motif of Gly-X, where X can be any of the amino acids alanine, serine, tyrosine, or valine (Varanko et al. 2020). Due to their high order structure, silk fibers show ductility, high toughness, and also they are biocompatible and biodegradable. The combination of all these properties makes them ideal candidates for use as high-performance structural components used for biomedical applications. There are also regenerated silk materials that are chemically reconstituted from natural silkworm cocoons to eliminate the binding proteins, sericin, and dissolve silk fibroin solutions to obtain hydrogels and resins (Nagarajan et al. 2019). β -sheet is the main structural component of silk fibroins. Native silk proteins have crystalline domains that are highly repetitive and are separated by crystalline or amorphous areas. The crystalline portion of *B. mori* fibroin has a repetition of 59 amino acids [GAGAGSGAAG[SGAGAG]8Y], with a 3:2:1 ratio of glycine, alanine, and serine (Tsuda and Suzuki 1981). Using the repeating motif [GAGAS], Capello and coworkers found that silk-like proteins (SLPs) developed crystalline structures that resembled the β -sheet structures of native silk proteins. Additionally, they discovered that blocks of amino acids like GAAGY can be used to adjust the degree of crystallinity. In their study, it was mentioned that the incorporation of SLPs with elastin-like blocks affects the crystallinity and also SELPs are more water soluble since the ELP blocks can influence the protein's molecular chain properties (Cappello et al. 1990). The thermal stability and adaptable mechanical properties of SLPs make them utilized for the formation of hydrogels and scaffolds. Recombinant silk-like peptides have also been used for biomedical applications.

One limitation is the level of expression when producing silk in large quantities. The most extensively used expression host, *E. coli*, cannot efficiently translate the highly repetitive gene sequence of silk protein. High levels of expression (2% of all soluble protein) were shown by transgenic species like potato and tobacco (Hood and Jilka 1999).

Elastin-like polypeptides: This class of polypeptides comprises of a genetically engineered kind of tropoelastin-derived protein polymers. They are genetically

encodable biopolymers, in the class of elastomeric proteins present in living organisms. Elastin is among the profuse extracellular matrix protein, in addition with collagen and glycosaminoglycans (GAGs). Environmental factors like temperature, pH, or ionic strength can cause them to go through a reversible phase shift. ELPs are soluble at temperatures below the inverse phase transition temperature and insoluble at temperatures beyond this threshold. The ability of ELP-based biomaterials to generate coacervates in response to temperature variations is one of their most important properties.

They are responsible for giving elasticity to tissues. Elastin-like polypeptides (ELPs) consist of hydrophilic and hydrophobic cross-linking domains. ELPs mostly consist of repeat units, Val-Pro-Gly-X-Gly, where X may refer to be any amino acid. At the fourth residue, amino acid proline is mostly avoided because it may interfere with coacervation (Wise et al. 2014). Different biofunctional groups can be incorporated into ELPs without affecting their thermal behavior. Recombinantly synthesized ELPs are genetically encoded, and they produce a monodisperse solution which is suitable for biomedical applications in contrast to synthetic polymers (Chilkoti et al. 2006). ELPs are very useful for biomedical applications since the phase transition temperature can be specifically programmed. Phase transition temperature is the maximum value at which ELP aggregation occurs (Fig. 1). Second, their molecular mass can be tuned when employed as drug carriers since molecular mass influences the half-life of the drug in the body, third, their expression level is high (typically expressed in *Escherichia coli*) compared to synthetic polymers, and also they can be purified by reverse phase transition without employing chromatographic methods (Kowalczyk et al. 2014). A very fine control over the sequence and length of ELPs can be beneficial for the biomedical application since depending on the above two, the chemical reactivity, biological activity, and physical properties of ELPs can be manipulated. Due to their broad range of applications, they have widespread use in drug delivery and tissue engineering.

A good understanding of the requirements and conditions of the amino acid sequence is necessary to design ELPs at the genetic level. Urry and coworkers studied by estimating the free energy change involved in the transition of ELPs containing a single guest residue, the effect of amino acid guest residues on the

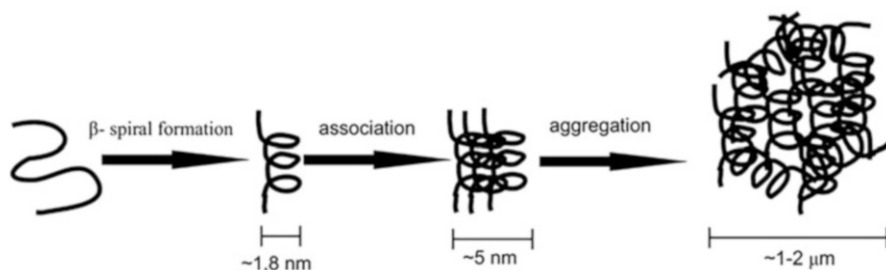


Fig. 1 The ELP aggregation mechanism. (The image is reproduced with permission from Kowalczyk et al. (2014) Copyright © 2014)

transition temperature, and the impact of hydrophobicity on the transition temperature of ELP (Urry 1997).

Synthesis

Polypeptides represent a large class of macromolecules comprised of amino acid sequences. Generally, their synthesis and design can be divided into main classes: the synthetic approach methods and the recombinant DNA techniques. In the synthetic techniques mainly include solid phase polypeptide synthesis (SPPS), ring-opening polymerization of α -amino-N-carboxyanhydrides (NCAs), and native chemical ligation (NCL). These techniques are based on the use of peptide monomers and nonpeptide monomers for the design of hybrid structures. A limitation to this method is the lack of control over polymer chain length and stereochemistry. Whereas recombinant DNA techniques are genetically encoded approaches toward the synthesis of polypeptides, through this method we can obtain highly specific sequence and stereochemistry in the synthesized polypeptide. The main drawback is that some of them cannot be adequately expressed into a heterologous host, and nonpeptide moieties can only be added after postexpression modification procedures (Duro-Castano et al. 2014).

Synthetic Techniques

To obtain polypeptides with comparable control of size and uniformity as those of genetically encoded approaches, there has been tremendous work taking place. Through this method, there is the possibility of incorporation of artificial amino acids, β -amino acids, and stereochemically unique amino acids all of which can prevent protease degradation. The main advantages of this technique include the following:

- (a) A vast number of unnatural amino acids can be employed for the synthesis to obtain different polypeptides with varying structure-function relationship and properties.
- (b) Large-scale synthesis is possible.
- (c) Easier and faster methodology compared to genetically encoded techniques.
- (d) Moreover, it enables the blending of polypeptides along with other synthetic polymers (Duro-Castano et al. 2014).

Solid Phase Peptide Synthesis (SPPS) as a procedure for preparation of polypeptides was first introduced by Merrifield in 1963 (Merrifield 1963, 1969). It consists of multiple coupling-wash-deprotection-wash cycles. In his work, Merrifield attached the first N-protected amino acid group to a partially chlorinated polystyrene resin through an ester bond. The protecting group was further removed to yield a free amino group that can react with next N-protected amino acid. The peptide is immobilized onto the solid phase and filtered. The resin is finally removed leaving behind the synthesized peptide. This method is highly advantageous since it

allows the synthesis of natural peptides, and certain control over peptide composition is achieved (Duro-Castano et al. 2014).

Native Chemical Ligation involves the chemoselective reaction of two unprotected peptide moieties diminishing the possibility of other side reactions and undesirable by-products. They form a nonamide bond at the ligation site.

Ring-opening polymerization technique is commonly used to produce peptides and polypeptide block copolymers. Due to the multiple functionality, structural homogeneity can favor self-assembly to define nanostructures that can find applications in biomedicine and biotechnology (Duro-Castano et al. 2014).

Genetically Encoded Polymers

The procedure basically consists of three steps:

- (a) A recombinant gene segment that codes for the desired protein is created. More specifically, a protein target is found, and its genetic code is translated.
- (b) Inserting this segment onto a plasmid from bacterium that is a DNA vector to create a recombinant DNA molecule.
- (c) The recombinant DNA molecule is transformed into a host cell.

The recombinant DNA molecule is subsequently used to successfully alter cells, which are then cultivated in culture. The accomplishment of recombinant genes is the rate-limiting step when the synthesized polypeptide chain has a large number of repeats.

On an automated solid phase synthesizer, DNA sequences up to 100 nucleotides can be chemically directly synthesized. The assembly of the gene into the desired vector is the rate-limiting step in genetically encoded synthesis (Fig. 2).

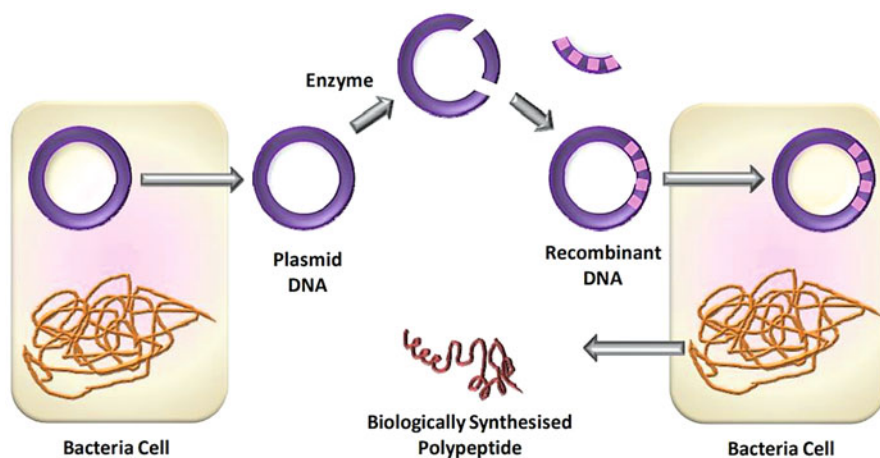


Fig. 2 Recombinant DNA synthesis of peptides. (Reprinted with permission from Duro-Castano et al. (2014))

Characterization Techniques

The biophysical, structural, and rheological characteristics of peptide-based materials can be ascertained using a variety of methods that were initially created to evaluate the solution properties of peptides and proteins and the mechanical properties of hydrogels.

Biophysical Properties

Numerous characterization methods that were first created for the characterization of proteins can also be used to characterize repeated peptides. It is possible to quickly assess the protein concentrations of peptide-based biomaterials using spectrophotometry, colorimetry, a Coomassie brilliant blue assay, or bicinchonic acid assay. These materials' molecular weights can be determined using analytical ultracentrifugation based on sediment coefficients, electrophoresis-based techniques like SDS-PAGE, mass spectrometry (MS) techniques like matrix-assisted laser desorption ionization (MALDI-MS) and electrospray MS, or electrophoresis-based techniques like SDS-PAGE. The lower critical solution temperature (LCST) or transition temperature (T_t), which is frequently calculated by the turbidity (optical density at 350 nm as a function of temperature), is typically used to indicate the inverse temperature transition behavior for ELPs.

The binding behavior of peptide-based biomaterials can be quickly and conveniently ascertained using surface plasmon resonance spectroscopy (SPR). Using a simple conjugation chemistry, such as 11-mercaptohexadecanoic acid, peptides can be easily immobilized on a gold-coated glass chip (MHA). It is possible to examine the binding thermodynamics of peptides with their binding partner using isothermal titration calorimetry (ITC). Calorimetric data can be used to calculate thermodynamic parameters such as binding enthalpy, entropy, free energy, and binding constant.

Structural Properties

Protein purification, tissue engineering, and drug delivery applications all depend on structural characteristics like hydrodynamic radius and appropriate folding. The best techniques for discovering detailed structural information about proteins are nuclear magnetic resonance (NMR) and X-ray crystallography. Circular dichroism (CD) spectrometry, which recognizes the unfolding and folding transitions of peptides, can also be used to study protein folding.

To specifically assess the proportion of alpha helices and beta sheets in a polypeptide, CD examines the absorption of left- and right-handed circularly polarized light. The functionality of peptide-based biomaterials, particularly those having enzymatic and biological capabilities, is significantly influenced by protein folding. Understanding the folding and refolding characteristics of peptides during chilling

and heating is possible with the help of differential scanning microcalorimetry (DSC).

The state of protein folding can be ascertained by measuring the amount of heat needed to raise the temperature of the peptide during a physical transformation, such as a phase transition, using DSC. The hydrodynamic radius (R_h) of polypeptides in aqueous solution can be calculated via dynamic light scattering (DLS), sometimes referred to as photon correlation spectroscopy. In DLS, an optical beam is sent through an aqueous sample, and the scattered light from the particles travels in all directions.

The measured scattered intensity changes over time due to constructive and destructive interference of the light scattered by nearby particles, which occurs because the particles are small enough to undergo random thermal (Brownian) motion. The diffusion coefficient of the particles can be determined by analyzing the intensity time correlation curve. DLS is particularly helpful for investigating how self-assembled polypeptide micelles are formed.

Rheological Properties

Since peptide-based biomaterials' main function is as a structural element to support cell growth, their rheological qualities are particularly crucial in tissue engineering scaffolds. By performing oscillatory rheological characterization in a solution state, it is possible to assess the load-supporting capacity of cross-linked or self-assembled polypeptides.

Another significant rheological metric for elastin-like polypeptides is gel swelling, which may be quantitatively characterized by the swelling ratio. When the swelling ratio is larger than one, the gel is said to be expanding, and when it is less than one, it is contracting. The swelling ratio can be quickly determined by weighing the peptide-based gels before and after incubation with buffer and then normalizing the measured weight to the initial weight.

Applications

Biopolymers have always been categorized as an efficient material for biomedical applications due to their properties like biocompatibility, biodegradability, low toxicity, and also the presence of biomimetic chemical entities, and they release no toxic elements upon degradation which is an important factor during human body metabolism. Hence they are widely used in the formulation of drug delivery vehicles, tissue-engineering scaffolds (Nagarajan et al. 2019). Peptide-based biopolymers are also utilized for biomedical applications in the field of therapeutic molecule delivery, tissue engineering, and food-packaging applications. Peptides or protein-based materials possess advantages like low cytotoxicity, abundant drug binding sites, and significant uptake to the targeted cells. Also owing to the different multiple functional groups in the amino acid sequence of polypeptides, they can be tuned to

create different interactions with the therapeutics forming a 3D network (Elzoghby et al. 2012).

Drug Delivery

Therapeutic drug delivery vehicles require a porous morphology to allow fast diffusion across the body to the target site. Peptide-based biomaterials have improved properties in terms of in vivo drug delivery and efficacy. There are several advantages of polypeptide over synthetic polymers since they are made up of amino acids which are biocompatible, and their metabolic products can be eliminated through natural metabolic process. Also most of the genetically encoded polypeptides have uniformity in sequence and molecular weight which can contribute to a great extent in properties like transport, biodistribution, and degradation (Das et al. 2019). Similar to synthetic polymers, amphiphilic polypeptide can encapsulate drugs into structures like vesicles and micelles. They also have the added advantage of reducing systemic toxicity, increasing plasma half-life, and increasing the solubility of poorly soluble drugs. The wide variety of functional groups, within the primary sequence of polypeptides, can be tuned for different interactions with the drug entity, also capable of forming a 3D network that can provide protection, targeted delivery and a reversible binding (Hoare and Kohane 2008).

Peptides have been reported to show great results in targeted drug delivery using peptide-modified polymer systems. The efficacy of the drug can be increased by delivering them directly to the target cell. It can also reduce adverse reactions and dosage. There are many reports in which these biomolecules are used to develop hosts of drug delivery platforms as such in drug depots for delivery of therapeutic drugs, injectable particles, small molecule drugs, and nucleic acids. Controlled drug delivery was first envisioned by a Nobel laureate, Paul Ehrlich, in the twentieth century who thought of a magic bullet therapy that could deliver drugs to a the desired target while also avoiding off-target toxicity. Collagen was among the first protein-based material used as a drug release system to treat Glaucoma (Varanko et al. 2020). Furthermore there have been many developments in the field of drug delivery using peptide-based biomaterials that can even help in cure of different types of cancers (Bertsch et al. 2019).

A quorum-sensing peptide from *Clostridium acetobutylicum* that had been previously discovered to pass the blood brain barrier was used by Ran and colleagues in their system (BBB). In order to deliver the chemotherapy drug paclitaxel to glioma cells, their technique utilized mPEG-PLA copolymer micelles modified with the D-retroenantiomer of this peptide produced from bacteria. Since the D-retroenantiomer has improved stability and glioma-targeting capabilities, the retro-inverso isomerization approach was applied to create their peptide. The usage of their mice model successfully targeted brain cancers (Tibbitt et al. 2016). The chemotherapy drug docetaxel was successfully loaded into a biodegradable system by Logie et al. to target breast cancer cells. They made use of micelles of poly (D,L-lactide-co-2-methyl-2-carboxytrimethylenecarbonate)-graft-polyethylene glycol (P(LA-co-TMCC)-g-PEG)

that had been altered with a brand-new taxane-binding peptide and linked to the antibody 73JFab. It was found that their peptide increased the drug loading about twofold, which indicated that this novel peptide can greatly enhance the capacity and, therefore, the efficacy of the delivery system (Logie et al. 2017). In a work carried out by a group of people, they developed O-carboxymethyl chitosan nanoparticles (O-CMC NP) and reformed them with a peptide that targets CXCR4 receptors that are able to deliver docetaxel to lung cancer cells. The reports showed that their targeted system performed significantly better than a nontargeted control at inducing apoptosis and causing cancer cell death (Wang et al. 2015).

Peptide-based nanoparticle devices are explored as potential drug and gene delivery vehicles for controlled release and delivery to the target site. A controlled release pattern is required for treatment of various diseases like malaria. Bhadra and coworkers created nanocarriers using poly-L-lysine-based dendrimers that were coated with chondroitin sulfate A (CSA) and PEGylated in order to deliver an antimalarial medicine called chloroquine phosphate in a regulated and sustained manner (CQ). The drug's cytotoxicity and hemolytic activity were significantly reduced after being entrapped in this peptidic carrier. Additionally, compared to the free drug, a significant decrease in the levels of the ring and trophozoite stages of *Plasmodium falciparum* was seen after treatment with drug-loaded CSA coated dendrimers (Bhadra et al. 2006).

Tissue-Engineering Applications

Peptide-based biomaterials are extensively used in regenerative medicine since they controlled degradation rate in vivo, are chemically biocompatible in vivo and in aqueous solutions, minimal cytotoxicity, the by-products are amino acids that can be easily eliminated from the body since they have controlled. Also, they can be functionalized to have better interactions with cells, thus providing an optimal platform for tissue functions and cellular activities. There have been several reports regarding the use of peptides and polymers for tissue-engineering applications mainly as implant scaffolds and for promoting tissue growth. For use in this field, the peptides need not be specific for a certain cell type (Okamoto and John 2013). The peptides will interact with components found in all cell types to increase or enhance interaction with cells; this property makes it ideal for design of scaffolds used in vivo and in vitro. The liquid-like precursors are handled and injected directly into the locations of the tissue defects before use. The polymers quickly come together after injection to produce a hydrogel that can act as a 3D artificial extracellular matrix. There are essentially three ways for polypeptides to gel: (1) by self-assembling in response to environmental stimuli, (2) by crosslinking, and (3) via physical cross-linking procedures (Chow et al. 2008; Hosoyama et al. 2019).

Garty and coworkers synthesized a polyethylene-oxide and polypropylene-oxide block copolymer thermoresponsive gel modified with Arginine-Glycine-Aspartate (RGD) peptides. It was reported that the gel exhibited low viscosity properties at ambient conditions that is suitable for direct injection at the targeted site. On

increasing the temperature, polymerization occurs which enables the formation of a rigid, supportive matrix that can restore function to damaged tissues and organs through previously embedded engineered stem cells (Ward and Georgiou 2011). Tissue-engineering scaffolds restore the function of diseased, injured, or aged cells, tissues, and organs. Self-assembling peptide and polypeptides can form hydrogels with well-defined nanostructure, 3–10 nm diameter fibrils. Zhang and coworkers have synthesized short oligopeptides comprising 12–16 amino acids that can form stable β -sheet structures which have been used to form self-assembled hydrogels that can support cell attachment to a variety of mammalian cells. These ionic oligopeptides have amphiphilic character with the charged residues, on the one hand, and nonpolar residues, on the other. The structures are very stable since they are formed by strong intermolecular interactions, ionic interactions, and Van der Waals interactions (Zhang 2003). Kisiday et al. formed hydrogel from oligopeptides containing leucine, lysine, and aspartic acid that supported the growth of chondrocytes. These hydrogels have also been utilized for cartilage tissue repair (Zhao et al. 2010) (Fig. 3).

Tissue Growth: ELP based polypeptide substrates have focused on cartilage repair as Betre et al., reported that above transition temperature, a gel-like material formed by aggregated ELPs can encapsulate chondrocytes which can maintain their distinctive morphology and phenotype in vitro for about 15 days which concludes that ELP aggregates supported the growth of chondrocytes. The cells could accumulate the characteristic proteins of cartilage, sulphated glycoaminoglycan (GAG) and collagen type II (Betre et al. 2002).

Biosensing

It is equally important to view the biomolecules clearly for the diagnosis, prevention, and treatment of diseases. Clear observation and results are required for identification of the problem. Peptides are regarded as recognition elements in the field of

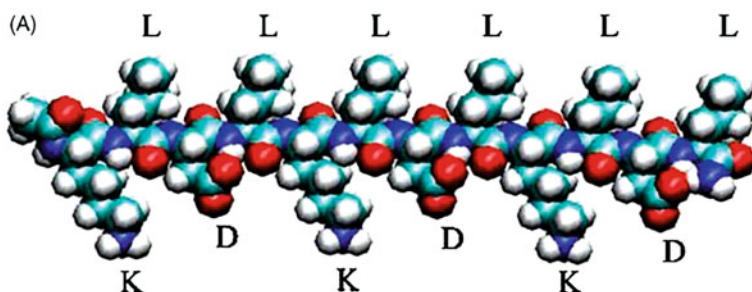


Fig. 3 Molecular model of a single β -sheet forming ionic oligopeptide positively charged Lysine (K), negatively charged aspartic acid (D) are on lower side of the sheet and hydrophobic Leucine (L) are on upper side facilitating self-assembly through intermolecular interactions (Zhao et al. 2010)

biosensing due to advantages like stability against denaturation, easy modification, chemical versatility, and cost effectiveness. Various peptide-based biosensors have been developed by conjugating a peptide with signal markers. They have been used for the detection of various analytes like cell proteins, nucleic acids, antibodies, and metal ions. They have also been used as probes in biosensors due to their property of forming several tertiary structures. There have been several reports on the use of peptide-based electrochemical biosensors for analytical approaches (Karimzadeh et al. 2018). Polypeptides have been utilized for the application of biosensors such as elastin-like polypeptides with a fusion protein A, G, or L that can bind to an antibody where it can act as a concentration mechanism to enhance signals (Gao et al. 2006). Also the biomolecules on the surface can be identified individually by immobilizing the biomolecules in a spatial controlled manner using local environment changes (Frey et al. 2003b). The reversible phase transition behavior of elastin-like polypeptides to regenerate the biosensing surfaces to create an actively controlled surface in which the surface properties such as hydrophobicity and functionality can be rapidly modulated through a simple temperature switch were reported (Frey et al. 2003a). Peptides being used as recognition nanoprobe were introduced by Kou and coworkers which utilized a peptide and DNA S_1 -modified platinum nanoparticles (P_1 -PtNPs- S_1) immobilized on electrode surface. They proposed this label-free electrochemical biosensor for sensitive detection of metalloprotease -7 (MMP-7). The peptide with the amino acid sequence (NH₂-KKKRPLALWRSCC-SH) provided a MMP-7 cleavage place (Kou et al. 2016). Proteases are enzymes that are involved in tumor growth and progression at primary and metastatic sites. One of the members of the serine protease family, trypsin, has been considered as a specific cancer biomarker. A work was reported on a low-cost, accurate biosensing method based on peptide-microbeads for the determination of trypsin. Here they have immobilized a short synthetic peptide sequence that is dually labeled with fluorescein isothiocyanate (FITC) and biotin onto neutavidin-modified magnetic microbeads. This biotin-modified peptide probe on the surface of magnetic microbeads further cleaves the target enzyme and enzymatic labeling. The biosensor showed good specificity against other proteins and proteases found in samples (Cristina et al. 2020).

Protein Purification

Recombinant proteins must be readily available for their numerous biological uses as medicines and diagnostics, as well as in regenerative medicine and biosensing. Proteins are essential tools for high-throughput screening, lead identification, and drug discovery. Even though recombinant expression allows for the mass production of numerous proteins, the cost of the finished product, which is primarily (70%) attributed to purification, might occasionally be too high for routine use. Additionally, it is still challenging to produce many additional proteins in sufficient quantities for biopharmaceutical applications, mostly because of issues with protein expression and purification.

These restrictions largely also apply to peptide-based polymers. Numerous methods have been created to enhance protein expression, and they have all been covered in-depth in other reviews. It is usual to use a variety of expression hosts, including bacteria, mammalian cells, and insect cells. The choice of these hosts is mostly determined by the characteristics of the proteins that will be produced. The requirement for appropriate folding and posttranslational modifications, which control the bioactivity of the purified protein, also influences the decision. Once the expression host has been chosen, the expression parameters, such as medium composition, feeding schedule, and metabolic waste disposal, must be optimized in order to maximize the protein production.

Controlled Drug Delivery to Tumors

Numerous investigational medicines have demonstrated effectiveness *in vitro* but have insignificant *in vivo* efficacy. Transport issues are typically to blame for this performance disparity; many medicines have poor pharmacokinetics or systemic toxicity, which limits their efficacy. Therefore, improvements in drug delivery are required to enhance the pharmacokinetics of possible treatments for numerous diseases, and peptide-based biomaterials hold considerable promise for *in vivo* drug administration. The strategies for delivering cancer therapies to solid tumors are the main topic of this section. Paul Ehrlich effectively invented the field of targeted medication delivery when he suggested the use of a carrier capable of delivering “therapeutically active groups to the organ in question” in 1906. In this method, drug carriers are created to increase the medication’s accessibility at a particular site while reducing systemic exposure to the drug. By directing the drug to a particular place, this approach provides an enhancement over the delivery of free medication. The drug’s effectiveness is raised, and the buildup at off-target locations is reduced, which reduces the compound’s toxicity.

However, targeted medicine delivery must get past the intricate transport obstacles in the body. Three transport phases can be identified in the process of delivering substances throughout the body: from injection to tissue location; from vasculature to interstitium; and from interstitium to molecular site of action. Additionally, depending on the site of action, medicines that have subcellular targets (such as the nucleus or lysosome) will need a fourth transport step. When a medication is taken systemically, the substance will build up at the site of action, but it will also build up in healthy tissue and may also be removed by the reticuloendothelial system at the same time (RES). Studies showing homogeneous distribution throughout the body imply that low MW medicines will quickly diffuse from the bloodstream into healthy tissues. The majority of the adverse side effects related to systemic therapy are brought on by the homogenous localization of the drug, which results in low drug concentrations at the tumor site. Small compounds are also quickly excreted from the bloodstream, necessitating repeated injections to exert therapeutic effects. The physiology of tumor vasculature differs from normal vasculature, which is one characteristic of tumors that can be used in medication administration. Normal

blood arteries have modest permeability, whereas tumor vasculature is asymmetrical and has leaky areas, which raises the overall permeability. The interstitium of the tumor also lacks functioning lymphatics. The enhanced permeability and retention (EPR) effect, which is a result of these two characteristics of tumors, increases the uptake of blood-borne chemicals into the tumor.

Gene Delivery into Mammalian Cells

Poly(amino acid)s, which include polypeptides and peptides, can be used as a functional material to create a variety of value-added materials, such as stimuli-responsive, self-assembling, nanoscale, and self-healing materials. Target-specific transport of bioactive chemicals into plant/animal cells and organelles is another illustration of how peptides are advantageous. Such delivery systems would be even more useful if they were biodegradable, biocompatible, and mechanically resilient. They would also be more advantageous if they could be made and processed in ambient water conditions without losing the bioactivity of the molecules that need to be delivered.

Because the resilience and stability of poly (amino acid)s may be altered by changing their amino acid sequences and secondary structures, such as β -sheet and α -helix structures, they have the potential to satisfy these requirements. Currently, this kind of material does not lend itself to chemo-enzymatic peptide synthesis. Due to its self-assembly, mechanical toughness, processing flexibility, biodegradability, and biocompatibility, silk, a structural protein, can assist in meeting these criteria and offers significant value for a variety of human therapeutic interventions.

Block copolymers made of spider dragline silk have been created and genetically engineered for application in tissue engineering and the delivery of genes. Due to their ability to destabilize membranes, condense DNA, and buffer pH, cationic components can be added to silk-based copolymers to deliver genes effectively. Cell-penetrating peptides (CPPs), for example, are 10–50 amino acid sequences that differ greatly in their sequence, electrical charge, hydrophobicity, polarity, and ability to translocate through membranes. Molecules and chemicals can be delivered to particular targets using signal peptides and antibody fragment sequences.

Gene Delivery to Specific Organelles

At different stages of life, organelles play crucial roles in cellular metabolic pathways. To build artificial metabolic pathways, it is essential to comprehend the core mechanism of cellular activity known as trafficking of biological molecules between organelles. However, the limitations of current organelle transfection techniques preclude both the use of organelles as a reaction site for the production of value-added biomolecules and the knowledge of the function and role of organelle genes. The double membrane, small size, and remarkable mobility of mitochondria, in

addition to their huge population size in cells, present significant obstacles to mitochondrial transfection. It has not yet been possible to successfully introduce foreign genes into the mitochondria of whole, vascular plants.

Conclusion

Peptide-based biomaterials have been reported to have immense number of applications in the biomedical field due to their unique properties and biocompatibility. Special emphasis has to be made on the detailed study of the design principles and structure-property relationship of the peptides and sequence of amino acids. The self-assembling of peptides to α -helix or β -sheet structures enable them to form unique nanostructures. Although most of the peptide-based materials enhance growth of specific cell types, more works can be done to make it more suitable for in vivo tissue-engineering applications. More techniques are required for identification and peptide modification and attachment to polymers. There are some limitations that are still causing problems like the thermal stability of peptides that can arise from aggregation, charge distribution, or ambient conditions. A clear picture of the design and properties of different peptides can make these biobased peptides a promising material for applications in the biomedical sector.

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Cross-Linking Biopolymers for Biomedical Applications

40

Anu Mary Joseph and Benny George

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Abstract

Biopolymers are preferred materials for medical applications on account of the great biocompatibility they exhibit. Their natural origin makes them highly bioactive and eco-friendly. But poor technological properties and quick deterioration rate pose enormous challenges on their performance and utility in proposed applications. The issues associated with lack of desired mechanical properties and aqueous stability of biopolymers can be managed to a great extent through cross-linking. Cross-linked biopolymers are found to behold great potential for biomedical applications like tissue engineering, drug delivery, and wound healing. Although, cross linking enables the biopolymers to meet the prerequisite of mechanical strength for various applications, it negatively interferes with other natural properties that made these biopolymers highly beneficial for biomedical applications. The reduction in degradability associated with cross-linking will have a negative impact while fabricating biomaterials for implants and drug

A. M. Joseph
Department of Chemistry, Newman College, Thodupuzha, India
e-mail: dranumaryjoseph@newmancollege.ac.in

B. George (✉)
Rubber Technology Division, Rubber Research Institute of India, Rubber Board, Kottayam, India

delivery systems in general. On the other hand, this negative side effect is extremely necessary and beneficial when longevity of implants or prolonged duration delivery of the pharmaceutical agent has to be ensured. Thus the equilibrium between mechanical strength and degradability must be decided during cross-linking biopolymers for their efficient functioning. Among the various options for biopolymer cross linking, the highly favored chemical cross-linking comes along with the grave disadvantage of cytotoxicity. In this regard, finding sustainable methods of cross-linking and newer cross-linkers are thrust areas of concern in order to ensure desired properties to biopolymers for medical applications. This chapter discusses about the main biopolymers, their cross-linking strategies, and the biomedical applications of cross-linked biopolymers.

Keywords

Biopolymers · Cross-links · Biomedical · Applications · Tissue engineering · Scaffolds · Drug delivery

Introduction

Mankind have been using polymers since long time by virtue of their natural characteristics and properties to meet the primitive requisites of ancient times like employing natural plant gum as adhesive or natural rubber latex for water proofing. The ancients neither modified these biopolymers nor synthesized newer ones to improve their qualities to meet growing demands. Although natural rubber has been known for a long time, the mighty status it enjoys today in mobilizing the modern world is attributed to a patented modification process viz. vulcanization discovered by Charles Goodyear in 1844 which uplifted its status from a waterproofing material to a technological marvel that caters to infinite number of applications in diverse fields. The biodegradable biopolymers may be varying in their source of origin such as from plants, from microorganisms, or from animals as represented in Table 1.

The Major Biopolymers

Starch and cellulose (polysaccharides) (Fig. 1) are the most important biopolymers in the large carbohydrate family. Starch can be produced from corn, wheat, sorghum, potato, etc. through a variety of environment-friendly green processes. Linear amylose and branched amylopectin serve as the components of starch. The amylopectin may be of varying structures defining the different physical properties of starch. The properties of starch can be improved and modified through cross-linking whether it is self-cross-linking or using multifunctional reagents. Both chemical modification and cross linking play decisive roles in the characteristics of the final modified starch and hence the quality of product. Starches find immense role in food, paper, and other nonfood industries. Ethanol production from starch is yet another promising application of starch (Sun 2013).

Table 1 Classification of biopolymers based on source of origin

Source of origin	Biopolymers
Microorganisms	Polyhydroxyalkanoates (PHA) Bacterial cellulose Hyaluronan Xanthan, curdlan
Plants	Cellulose and derivatives Lignin Starch and derivatives Alginate Lipids Gums Poly lactic acid
Animals	Chitin Chitosan Hyaluronan (polysaccharides) Casein Whey Collagen Albumin Keratin Leather (proteins)

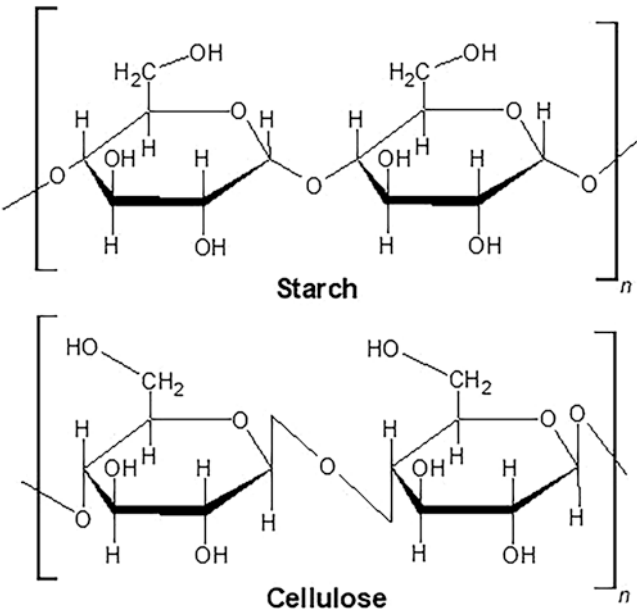


Fig. 1 Structure of starch and cellulose

Cellulose is a major constituent of natural fibers and is made up of linear polymer chains composed of anhydroglucose units. Cellulose chains form three dimensional network structures via hydrogen bonding between the chains which contributes

toward mechanical and chemical characteristics. Cellulose derivatives like cellulose acetate, cellulose acetate propionate, and cellulose butyrate find applications in the field of thermoplastics. Cellulose is used in biomedical applications like structuring controlled drug delivery devices, scaffolds for various regenerative applications, wound dressings, etc. (Yadav et al. 2015). Methylation of cellulose yields methyl cellulose, the most important and simplest cellulose derivative. Methyl cellulose finds immense applications such as tissue engineering, wound healing, and pharmaceutical formulations (Salihu et al. 2021). Carboxymethylcellulose (CMC) is another biodegradable and ordinary derivative of cellulose having hydrophilic carboxylate groups in its polymeric backbone. Its hydrophilicity and associated swellability make it an ideal candidate for preparing hydrogels (Mali et al. 2018). CMC has high tendency to form intramolecular cross-linking thereby lowering the mechanical strength of the fabricated products which is usually addressed combining with another cellulose derivative like hydroxyethylcellulose (HEC) to improve the intermolecular cross-linking. Esterification of hydroxyl groups of cellulose generates yet another class of commercially important thermoplastic cellulose derivatives called cellulose esters (Salihu et al. 2021).

Lignin is a biopolymer obtained from lignocellulosic biomass. Chemically, lignin is a randomly branched amorphous phenolic polymer cross-linked with cellulose and hemicellulose units. Lignin is obtained from common biomass through various processes and further extracted to obtain what is called technical lignin which is the usable form of lignin. As it is generally obtained as an unwanted by-product of agricultural, paper and pulp industries' lignin qualifies the economic and availability requirements for any reliable raw material for several applications. Besides, lignin possesses a high carbon content, considerable stability at processing temperatures, coupled with biodegradability and biocompatibility ensuring it to serve as a promising material for several applications. The industrial applications of lignin are guided by another driving force that it reduces the carbon footprint of a manufactured product. Lignin has been successfully employed in applications like asphalt and concrete preparation, antioxidant for polymer and cosmetic products, raw material for synthesis of carbon fiber, adhesive, foams, packaging paper industry, preparing different phenolic chemicals, dispersing agent, etc. The antioxidant, antitumor, antimicrobial, antidiabetic, and antiviral effects of lignin have been exploited by pharmaceutical industry (Spiridon 2018). Advanced scientific research has contributed greatly toward the vast and popular use of lignin composites for a handful of prominent biomedical applications such as in fabrication of scaffolds, dressing materials, delivery carriers, etc. (Terzioğlu et al. 2020; Yu and Kim 2020).

Alginate is calcium, magnesium, and sodium salt of alginic acid, and it is a biopolymer extracted from brown seaweed (Fig. 2). Alginate is a copolymer of guluronic acid and mannuronic acid in which both the monomers contribute greatly toward the toughness and elasticity of the polymer. Alginate possesses a wonderful combination of tackiness, gelling properties, and firmness all supplementing its industrial applications. Pharmaceutical industry favors it on grounds of its lower noxious potential, commendable biofriendliness, and possibility of easy gelation by divalent cations and low cost. The use of alginate hydrogels for tissue drug delivery

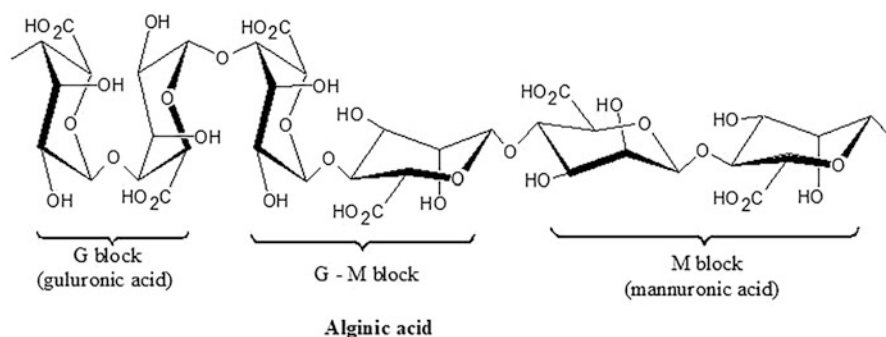


Fig. 2 Structure of alginic acid

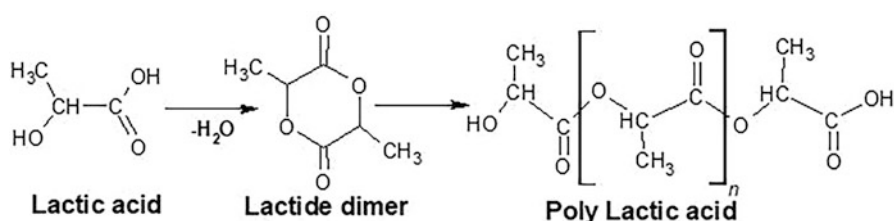


Fig. 3 Schematic representation of formation of poly lactic acid

is being widely used nowadays. Alginate simulates the structure and makeup of extracellular matrices of living tissues, making it the right choice for a wide array of biomedical applications like delivering bioactive agents, fabricating scaffolds for wound healing and cell transplantation.

Poly lactic acid (PLA) is a natural, eco-friendly, and compostable biopolymer obtained from sustainable sources like cornstarch or sugarcane. Bacterial fermentation of the plant raw materials yields lactic acid, which on dehydration gets converted to the lactide dimer which can be polymerized to high molecular weight polymer without further dehydration as represented in Fig. 3 (McKeen 2012). PLA, being a polymer, owes its technical properties to its molecular weight, crystallinity, and stereochemistry of PLA backbone (DeStefano et al. 2020). PLA is a thermoplastic of high strength and high modulus that can be used to fabricate products for industrial packaging or the biocompatible/bioabsorbable biomedical applications. PLA has been regarded as a promising biopolymer that caters to a handful of biomedical applications in various fields of healthcare such as tissue engineering, ortho and dental prosthetics, and medical tools/equipment/accessories owing to its excellent biodegradability into nontoxic products (DeStefano et al. 2020). Photocross-linked lactide copolymers in which PLA undergoes copolymerization of another monomer unit such as polyethylene glycol, tetramethylene oxide, caprolactone, polyglycolide, poly (trimethylene carbonate), etc. contribute to a

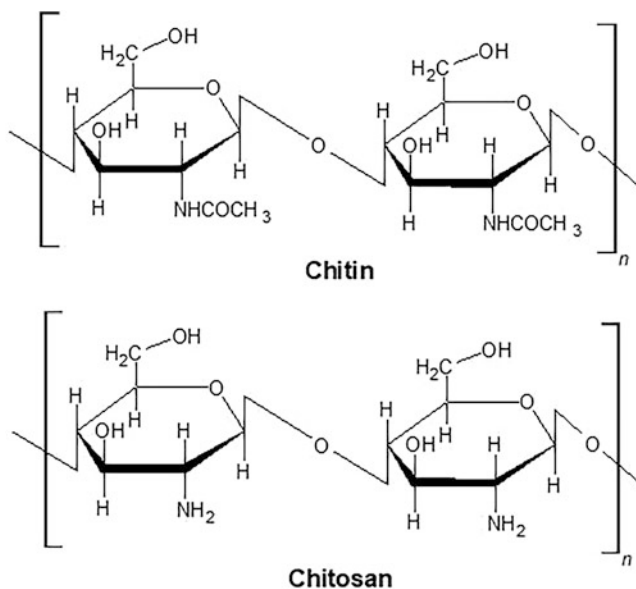
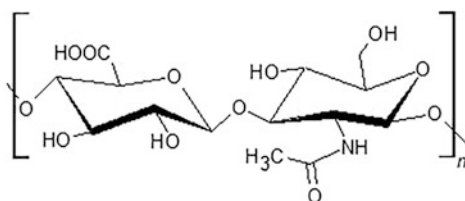


Fig. 4 The structure of chitin and chitosan

special group catering to the calls of biomedical industry as scaffolds for tissue engineering or drug carriers (Bednarek et al. 2020).

Chemical structure of chitosan can be described as a straight-chain copolymer of D-glucosamine and N-acetyl-D-glucosamine obtained through deacetylation of chitin. Chitin is the second most abundant natural polysaccharide and is commonly found in the exoskeletons of crustacean arthropods. The structures of chitin and chitosan are illustrated in Fig. 4. Since chitin is a waste from food industry, chitosan production is cost-effective and eco-friendly. Chitosan resembles cellulose in structure. The extent of deacetylation of chitin plays a decisive role in determining characteristics like solubility, biodegradability, reactivity, and adsorption capacity of a particular chitosan sample (de Alvarenga 2011). Generally, extent of deacetylation falls in the range 60–100% in commercial chitosans as determined by NMR spectroscopy. High antimicrobial activity provides a promising future for chitosan in food industry as preservative and packaging material while its biodegradable, biocompatible, antimicrobial, nontoxic, and antitumor attributes favor biomedical and pharmaceutical applications. Although chitosan is widely used for drug delivery and tissue engineering, its molecular weight has to be decided according to specific applications. High molecular weight chitosan is obtained through grafting it with synthetic monomers to produce cross-linked modified chitosan copolymers having improved physicochemical properties. Since the amino groups are responsible for the distinct characteristics, the characterization of the polymer in either chitin or chitosan (degree of deacetylation) is extremely important while deciding a possible industrial application. Chitosan finds immense

Fig. 5 Structure of the repeating disaccharide units of hyaluronic acid



Repeating disaccharide units of Hyaluronic Acid

applications in fields like photography, biotechnology, cosmetics, food processing, biomedical products pharmaceuticals, waste water treatment via adsorption techniques, etc. (de Alvarenga 2011).

Hyaluronic acid (hyaluronan, HA) is a linear polysaccharide biopolymer of N-acetyl-D-glucosamine and glucuronic acid (Fig. 5). The molecular weight is generally about millions of Daltons. HA is a component of almost all body fluids and tissues enabling its widespread applicability as an indicator for several diseases. It is also administered as intra-articular injections to augment defected synovial fluid in arthritic patients. Another thrust application area is in the fabrication of biomedical scaffolds. HA is greatly recognized for its wound-healing attributes. High molecular weight HA is naturally delivered to injured skin, where it initially helps to form clots by binding with fibrinogen and forms a gel medium for immune cells. Later, HA breaks down to molecular weights around 120 kDa, which promote inflammation – useful at that stage of healing – and blood vessel rebuilding. During final stages of healing, HA further breaks down to oligomers, which suppress inflammation and help proliferation of new skin cells and collagen.

Collagen, the prime structural matter of vertebrates and the most abundant protein in mammals is synthesized by fibroblasts and accounts for about 20–30% of total body proteins. Collagen molecule have a triple helical structure extended to rod-shaped structure with an average length of about 3000 Å and a width of about 15 Å to attain a molecular weight of 300 kDa approximately (Yadav et al. 2015). In addition to its nontoxic, biocompatible biodegradable nature, it possesses high tensile strength and water affinity, and its weak antigenicity ensures easy absorption. Collagen is considered as a highly useful biopolymer due to its low immunogenicity and high biocompatibility. On the other hand, due to the destruction of assembly structure and natural cross-linking during extraction mechanical strength, thermal stability, and enzyme resistance, becomes inferior hampering the biomedical potential of natural collagen, which can be circumvented through modifications like chemical, physical, or biological cross-links to minimize degradation thereby enhancing mechanical stability. Introduction of additional cross-links restricts collagen molecules from sliding past each other under stress thereby increasing its mechanical strength, improving stiffness and tensile strength, and reducing extensibility. Potential applications of cross-linked collagen in tissue repair and biopharmaceutics include wound dressing, tendon and joint repair, treatment of corneal and cardio-vascular diseases, tissue engineering, bone-tissue engineering,

neural regeneration, prosthetic heart valves and vascular prosthesis, drug/gene/cell delivery agents, scaffolds, etc. (Gu et al. 2018).

Gelatin is a water-soluble material derived via partial hydrolysis of collagen to disrupt the tertiary, secondary, and to some extent the primary structures. Gelatin is a protein (polymer) formed from a remarkable array of amino acids comprising of all the vital amino acids except tryptophan (Yadav et al. 2015). Gelatin has excellent biocompatibility, plasticity, and adhesiveness contributing to its ability to form foam and emulsions and moisturizing effects. Its inherent hydrogel nature of gelatin simulates extracellular matrix (ECM) extending its suitability for in vitro cell culture. Cross-linked gelatin matrices have been fabricated for different biomedical applications for tissue engineering and drug delivery (Xiang and Cu 2021).

Keratin is the cysteine-rich fibrous protein that assists in the formation of structures such as hair, horns, feathers, wool, and nails. The wide range of properties and structures accomplished by keratin can be related to the sulfur content in them. Hard keratins with a higher amount of sulfur cross-links form hard skin structures. Soft keratins with lower sulfur content are comprised of slack bundles of cytoplasmic filaments and serve to offer pliability to epidermis. Keratins are classified into alpha-keratins and beta-keratins based on their structure. Alpha-keratin consists of polypeptide chains arranged as alpha-helices while beta-keratin is formed by pleated beta-sheet arrangement of polypeptide chains. Keratin is extracted from keratin-rich materials through a wide variety of techniques like chemical (reduction, oxidation, alkali extraction, and ionic liquids), enzymatic, physical (steam explosion), or radiation exposure (microwave irradiation) methods (Feroz et al. 2020; Rouse and Van Dyke 2010).

Applications of Biopolymers

Biopolymers find immense applications in construction materials, automobile parts, furniture, packaging, utensils, printing, coatings, and related industries as products like adhesives, resins, plastics, binders, fibers, paints, inks, additives, solvents, etc. Plant polysaccharides find extensive applications in food industry as source of carbohydrates, monomer sugars, sweet syrups, sweeteners and dietary fibers, jelling agents, immune boosters, additives, thickeners, emulsifiers, gelling and texturizing agents, fat and/or sugar replacers, etc. Polysaccharide biopolymers, especially starch, are found to be well-suited raw material for biodegradable plastic production and nano-material science due to their self-assembly capabilities and stimuli responsiveness. Cellulose from wood pulp finds applications in the production of paper and cardboard, coatings, building materials, pharmaceuticals, foodstuffs, and cosmetics. The nano-materials derived from these biopolymers find immense uses as reinforcing elements in composite materials, biosensors, environmentally sensitive membranes, artificial muscles, solar materials, etc. The growing demand for sustainable biofuels depends mostly on polysaccharide biopolymers (like corn starch for biodiesel production) as the raw material (Poli et al. 2011).

Natural polymers are increasingly used for bone replacement and hard tissue augmentation. Natural polymers like collagen (proteins), chitosan, alginate, starch, cellulose (polysaccharides), etc. are widely employed for different pharmaceutical and biomedical uses owing to their nontoxic, biocompatible, biodegradable, and water-soluble nature. Biopolymers are mostly employed as delivery vehicles for drugs or bioactive agents, fillers in pharmaceuticals, dressing products, scaffold and implant components in tissue engineering, etc. Different biopolymer classes were found to be associated with various health benefits such as lowering blood cholesterol (pectins), lowering intestinal transit time, increasing stools bulk (dietary fibers), and anticancer, antioxidant, antibacterial, antiviral, and chemopreventive effects (several plant polysaccharides) (Poli et al. 2011). Such biopolymers were exploited for such beneficial roles in medical service to a greater extent.

Cross-Linking Biopolymers

The process of cross-linking has been a promising research realm as far as processing and applications of biopolymers are concerned. The very success of biomedical applications of these biomaterials is determined by the effectiveness and efficacy of cross-linking. The poor mechanical properties exhibited by biopolymers are mostly compensated through cross-linking them to form a firm network. A cross-link is a physical (interactions such as ionic bonding, hydrogen bonding, etc.) or chemical bond (covalent bonding) that connects the functional groups of different polymer chains creating a three-dimensional network with improved qualities. The agents (physical/chemical/radiation) used for cross-linking are termed as cross-linkers or cross-linking agents, and chemical cross-linkers that can form covalent bonds between different functional groups of the polymer chain are the most favored ones. Cross-linking affects polymer properties like tensile strength, rigidity, cell-matrix interactions, thermal stability, solubility, degradation resistance, shape memory retention, etc. (Balakrishnan and Jayakrishnan 2005). Tailored cross-linking enables modulating controlled release of encapsulated materials which is now a highly active research area in theranostics (Thakur et al. 2018). Multiple options are available for cross-linking biopolymers so as to fabricate them as scaffolds or delivery vehicles. The type and chemical nature of the biopolymer plays a decisive role in this respect.

Physical Cross-Linking

Physical cross-linking involves electrostatic interactions between polymer chains through ionic bonds forming a stable three dimensional network structures (Fig. 6). Such ionic bonds are formed in sodium alginate gels when exposed to calcium ions which involve bridge formation between alginate chains. When borax is added to polyvinyl alcohol, hydrogen bonds (weak interaction forces) are formed between boric acid and the alcohol groups of the polymer. Biomaterials like gelatin, collagen, agarose, agar-agar, etc. can undergo physical cross-linking. Generally, physical cross

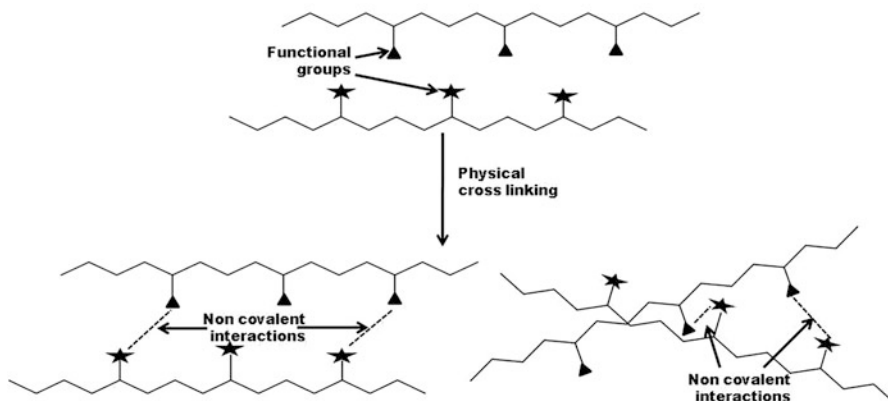


Fig. 6 Schematic representation of physical cross-linking

links are less stable both mechanically and thermally in comparison with covalent cross-links.

Cross-Linking via Irradiation

Exposure to ultraviolet, visible, or gamma radiations are the common irradiation methods of cross-linking. The major setback of such cross-linking is the absolute lack of control over the cross-link density attained by the polymer matrixes which in turn hamper with the release properties. Cross-linking biopolymers via irradiation-based techniques either make use of high-energy-ionizing radiation or photo-initiator molecules or are widely used for collagen scaffolds (Fang et al. 2020). Irradiating biopolymers with high-energy radiations trigger two competitive and mutually opposing processes, namely degradation via fragmentation of polymer chain and cross-linking (Fig. 7). Preserving the balance between the two processes is a critical factor that eventually influences the mechanical degradation behavior of cross-linked products. Photo cross-linking can also be carried out with visible light making use of photo initiators. Using trisbipyridyl ruthenium as a photo initiator to cross-link a chitosan hydrogel for bone regeneration suggests the feasibility of such an approach (Lin et al. 2018). Reports point to cross-linking collagen and silk fibroin-based biomaterials specifically for ophthalmic uses through ultraviolet radiation (Bhattacharjee and Ahearne 2021).

Dehydrothermal Cross-Linking (DHT)

Dehydrothermal cross-linking involves thermally induced dehydration leading to condensation reactions between monomers to generate polymeric networks (Fig. 8). In DHT, the reaction is driven forward by removing the by-product of cross-linking (water) applying heat and vacuum according to Le Chatelier's principle. Although this method is commonly used for synthesis of synthetic polymers like polyesters, polyethers, polyamines, polyamides, etc. via bonding two complementary functional groups by removal of water molecules through thermal stimulation, recent reports

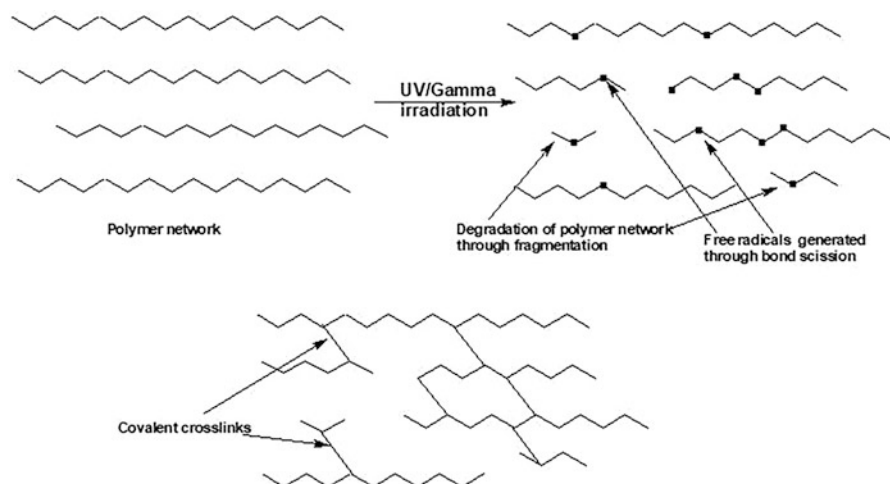


Fig. 7 Schematic representation of cross-linking via irradiation

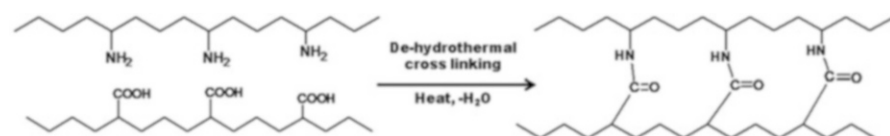


Fig. 8 Schematic representation of dehydrothermal cross-linking

suggest repetitive use of this method for cross-linking of the polymeric scaffolds (Nair et al. 2020).

Chemical Cross-Linking

Chemical cross-linking leads to covalent bonds between functional groups of biomaterials through common chemical reactions like acylation, carboxylation, esterification, alkylation, etherification, halogenation, and Schiff alkalization reactions (Wang et al. 2020). Chemical cross-linkers are either nonzero-length cross-linkers or zero-length cross-linkers. Nonzero-length cross-linkers are bifunctional or multifunctional molecules linking different polymer chains through different functional moieties. These cross-linkers bridge the functional groups such as free carboxylic acid groups, amino groups, and hydroxyl groups that present on the polymer chains so as to form cross-links between biopolymer chains. Glutaraldehyde, polyepoxides, and isocyanates are examples of nonzero-length cross-linkers. The zero length cross-linkers like acyl azides, transglutaminase, and water-soluble carbodiimides create covalent bonds by inducing chemical reaction between the reactive functional groups on the polymer chains itself. A schematic representation of chemical cross-linking is given in Fig. 9.

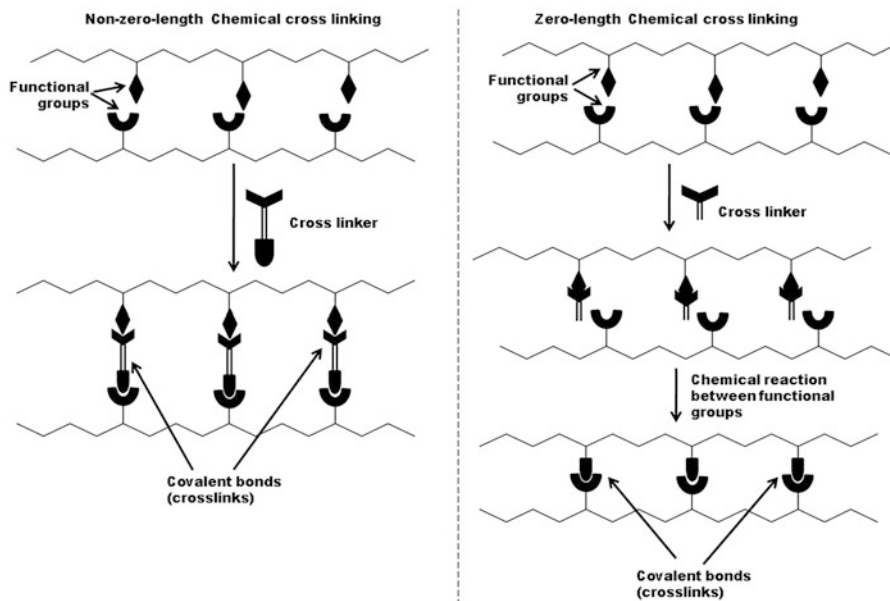


Fig. 9 Schematic representation of chemical cross-linking

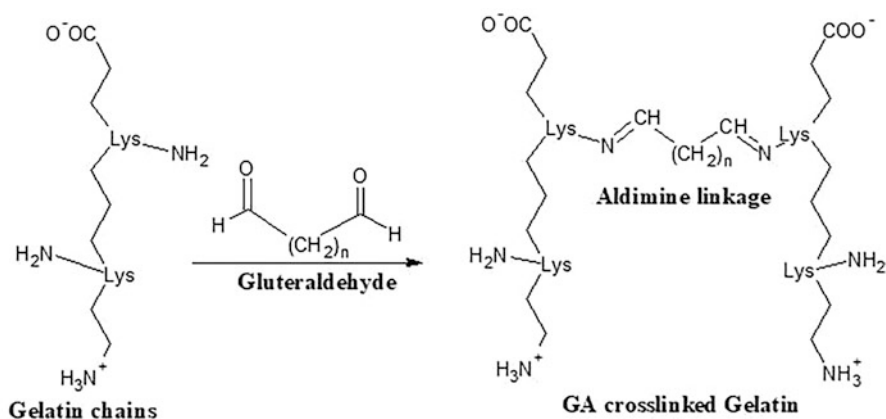


Fig. 10 Schematic representation of cross-linking gelatin chains using glutaraldehyde

The mostly employed chemical cross-linkers for biopolymers are briefly discussed below.

Glutaraldehyde (GA) has been a widely used chemical cross-linker for various biopolymers as it can appreciably advance the technical capabilities and service-life of biomaterials. GA acts on the amine or hydroxyl functional groups through a Schiff-base reaction to join the individual biopolymer chains through intra- or intermolecular interactions resulting in a cross-linked network (Fig. 10). Irrespective

of the fact that this method has been the gold-standard cross-linking method for many years, the cytotoxicity and biohazard problems induced by aldehyde functional groups of GA may give rise to rigorous inflammatory responses. These serious negative impacts led to a handful of detoxifying counter measures to ensure safety and reliability of GA-cross-linked biomedical products (Oryan et al. 2017).

1-Ethyl-3-(3-dimethyl aminopropyl)-carbodiimide (EDC) is the most commonly used carbodiimide for cross-linking biomolecules by virtue of its water-soluble nature and reactivity with carboxyl, hydroxyl, and sulfhydryl functional groups. EDC offers a noncytotoxic, zero-length, cross-linking alternative to GTA as the access is limited to “neighbouring” free amines alone (Nair et al. 2020). EDC serves as a suitable cross-linker for collagen scaffolds for numerous tissue-engineering applications. N-hydroxy-succinimide (NHS) is also used in such cross-linking reactions to activate carboxylic acid groups (Fig. 11) (Yang 2012). Although it possesses lower cytotoxicity than GA, it shows poor biomechanical properties and more rapid biodegradation profiles, in comparison with GA-cross-linking in most cases (Oryan et al. 2017).

Genipin is a natural, colorless, water-soluble cross-linking agent extracted from gardenia fruit (*Gardenia jasminoides* ELLIS). Geniposide is the parent compound from which genipin is obtained via enzymatic hydrolysis with β -glucosidase. Genipin is widely used in customary Chinese remedies owing to its antiphlogistic, anti-inflammatory, diuretic, choleric, and hemostatic properties (Butler et al. 2003; Oryan et al. 2017). Several studies point to its lower cytotoxic potential in

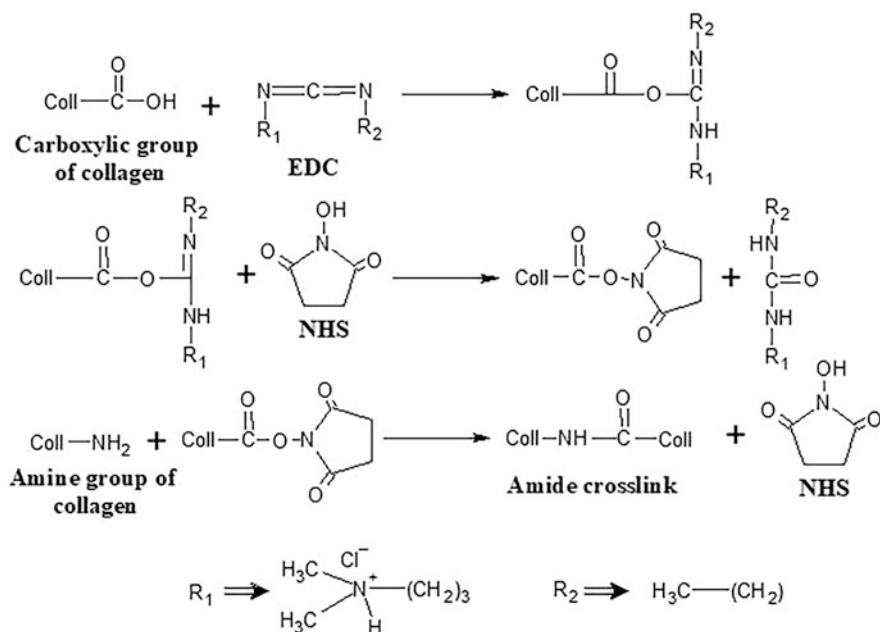


Fig. 11 Schematic representation of cross-linking collagen chains using EDC

comparison with glutaraldehyde. Hence, Genipin finds wide employability as the cross-linker while fabricating most of the biopolymers for various biomedical applications (Oryan et al. 2017). Genipin forms blue-colored hydrogels on cross-link formation with the amine groups of biopolymers like chitosan and gelatin. The most important applications of genipin include the fabrication of hydrogels as cartilage substitutes, carriers for controlled release of drugs and bioactive molecules, scaffolds for medication of wounds, etc. (Muzzarelli 2009). The cross-linking reaction pathway associated with genipin cross-linking has been investigated in detail, and two possibilities have been put forth. The possibility of an S_N2 nucleophilic attack by a primary amine group on the biopolymers that replaces the ester group on the genipin molecule by a secondary amide linkage to form a compound of genipin linked to the amine residue of the biopolymer is an option. Another possibility is an initial nucleophilic attack on the genipin C3 carbon atom by a primary amine group of biopolymer to form an intermediate aldehyde along with the opening of the six membered dihydropyran ring giving rise to a secondary amine. The amine thus formed will now attack on the aldehyde group to form heterocyclic compound of genipin linked to the amine residue of the biopolymer. The bifunctionality of genipin can ensure cross-linking between biomolecules via both these proposed mechanisms (Butler et al. 2003). Schematic representation of cross-link formation using genipin is given in Fig. 12 taking chitosan as a representative biopolymer.

Citric acid (CA) is yet another natural cross-linker for biopolymers found in citrus fruits. Its ability to react with both amine and hydroxyl groups qualifies citric acid as a promising cross-linking agent for polysaccharides and proteins biopolymers (Fig. 13) (Uliniuc et al. 2013). Besides, the residual pendent groups remaining

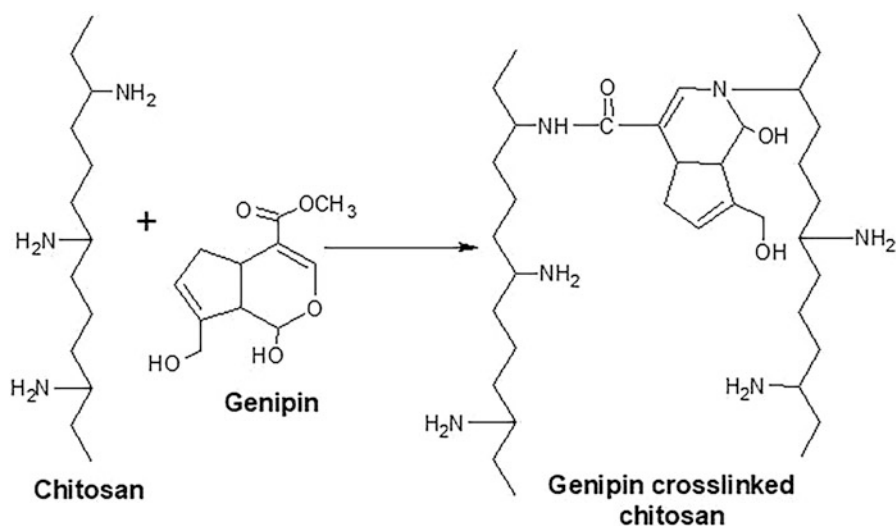


Fig. 12 Scheme for cross-linking chitosan with genipin

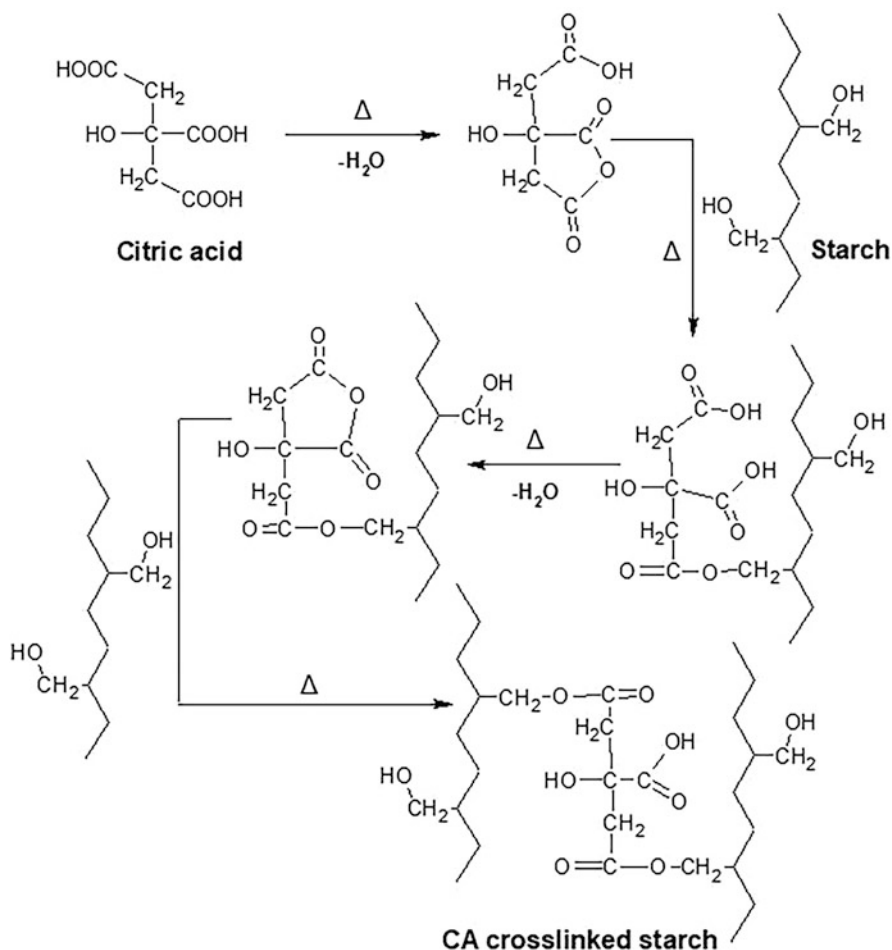


Fig. 13 Schematic representation of cross-linking starch using citric acid

after CA cross-linking of biomaterials can form ester bonds, which contributes positively toward hemo-compatibility, maintenance of hydrophilicity of the polymer network, and augmenting sites for bioconjugation (Mali et al. 2018).

Enzymatic Cross-Linking

Another well-sought approach toward modification and cross-linking of polymers makes use of enzymes to achieve cross-linking of biopolymer chains (Fig. 14) (Broguiere et al. 2018). Transglutaminase (TGase) is a common enzyme for most living organisms and is found to be associated with many biological activities like epidermal keratinization, blood clotting, and regulation of erythrocyte membranes and many human diseases (Parkinson's, celiac disease, and cancers). Enzymatic

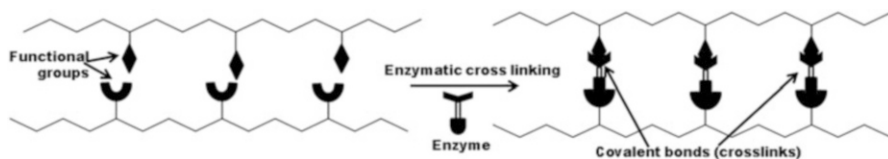


Fig. 14 Schematic representation of enzymatic cross-linking

cross-linking employing transglutaminases was used for diverse tissue-engineering applications with respect to increased cross-linking efficiency, feasible conditions, and commendable cyto-compatibility. Some of the transglutaminases are commercially available and FDA-approved cross-linkers on grounds of their nontoxic and nonimmunogenic nature. Enzymatic cross-linking using Tgase has also been explored for controlled drug release. The “substrate specificity” eliminates issues of side reactions in the case of enzyme cross-linking. Irrespective of all these advantages, the poignant influence of reaction conditions on the catalytic activity of enzymes shadows the widespread possibilities of enzymatic cross-linking for biomedical applications (Song et al. 2021).

Cross-Linking Biopolymers for Biomedical Applications

The greatest advantage of employing biopolymers for biomedical applications is their great biocompatibility. Their natural origin enables them to be highly bioactive and support normal body functions they are destined to perform. Eco-friendly nature of biopolymers is an added advantage in many of their biomedical applications. On the other hand, disadvantages like poor technological properties and rapid deterioration rate though hamper their performance, and application options might be overcome through proper chemical modification and cross-linking (Litowczenko et al. 2021). Tissue engineering is a potential research field that caters to replacement and repair of injured or unwell tissues and body parts with simulated substitutes fabricated from natural or synthetic materials. Prostheses, parts that assist tissue regeneration, delivery vehicles for drugs, cells or biomolecules, coating of non-biological devices with biocompatible materials, etc. (Vukajlovic et al. 2019) will all fall under the vast heading of tissue engineering. Need for improving the mechanical strength of biopolymers is a prerequisite for fabricating them for various applications. From biomedical point of view, however, it must be considered that cross-linking not only enhances mechanical properties but also interferes with other natural properties like degradability, solubility, gas permeability, etc. The reduction in degradability is a basic effect associated with cross-linking, and this might have negative impact while fabricating biomaterials for biomedical applications like implants and drug delivery systems. On the other hand, cross-linking is extremely necessary and beneficial when longevity of implants or delivery of the pharmaceutical agent has to be ensured for prolonged duration (Bednarek et al. 2020). Thus the equilibrium between mechanical strength and degradability must be decided during

cross-linking biopolymers so as to enable them to fulfill the requirements of the target application.

Biomedical Applications of Cross-Linked Biopolymers

The biopolymers are biocompatible and biodegradable which make them useful in applications, like drug transport materials, medical implants, wound healing, tissue scaffolds, and dressing materials in pharmaceutical industries. When the incompetency associated with mechanical properties is circumvented through cross-linking, these biopolymers provide ample raw materials for fabricating biomaterials according to various creative scientific and technological approaches. Cross-linked biopolymers are promising candidates for drug delivery, scaffolds for hard and soft tissue regeneration, wound dressing, grafts and implants for tissue replacement, cancer treatment, etc. The important ones are discussed in detail.

Scaffolds

Scaffolds are porous and degradable structures that are used to offer mechanical support to bone defects, and simultaneously permit unhindered proliferation and differentiation of surrounding cells (Vukajlovic et al. 2019). Bioscaffolds mean scaffolds made by using natural polymers or with the addition of active bioagents. Biocompatible and biodegradable polymer scaffolds are indispensable for tissue engineering and drug delivery (Balakrishnan and Jayakrishnan 2005). Scaffolds are expected to meet certain basic requirements: (a) Scaffold surface should permit cell adhesion and proliferation; (b) scaffold should not induce inflammation or an immune response (biocompatible); (c) degradation products of scaffolds cannot be cyto-toxic; (d) scaffold should closely simulate the native tissue it is trying to mimic with respect to structural and mechanical properties; and (e) scaffold should be porous enough to allow unhindered cell growth and nutrient diffusion (Dovedytis et al. 2020; Litowczenko et al. 2021). The most popular polymers for scaffolds fabrication are chitosan, cellulose, alginate, hyaluronic acid collagen, gelatin, fibrin, etc. which find potential applications in tissue engineering of skin, nerves, bone, cartilage, heart, liver, muscle, retina, vascular system, etc. (Kramschuster and Turng 2013).

Bone Tissue Regeneration

Among prostheses, orthopedic implants are in particular demand and bone transplantation is the second most common tissue transplantation all over the world. Tissue engineering has developed several synthetic bone graft substitutes especially hybrids. A hybrid is a composite of an inorganic and an organic material, whose properties are improved over the individual properties of the two materials through mutual interactions between them. Based on these interactions, hybrid materials are classified as Class I hybrids (weak bonds like electrostatic, van der Waals, and hydrogen bonds), Class II hybrids (covalent bonds). An initial attempt in this direction is collagen and calcium phosphate composites which are suited for dental

applications and repairing joints and broken bones. These composites share a similar composition as that of natural bone and tissue (inorganic phase and an organic phase) and can probably achieve better mechanical properties complimented by the strength of ceramics and flexibility of polymers.

Nerve Regeneration

Peripheral nerve regeneration is an intricate and a very old remedial dispute that requires proper leads for reconnecting neural damages and reinstating the proper state of nervous system. Researchers have exploited the possibilities of several polymers, both natural and synthetic to find the best candidate for constructing nerve conduits that can achieve desired nerve-regenerative functions. Natural polymers like chitosan, collagen, gelatin, hyaluronic acid, and silk fibroin are used to fabricate nerve conduits by virtue of their biocompatibility, absence of serious immune response, regenerative signaling without growth factors, and degradation by natural enzymes. However, quality-wise inconsistency demanding elaborate refinement and characterization requisites, low technological properties, and high degradation rates associated with biopolymers demands chemical modification, cross-linking, and blending with other structural components (Wang and Cai 2010) so as to fit them in for most of the applications.

Drug Delivery Systems (DDS)

The physiological responses of the body following its administration primarily decide the efficacy of a drug. Once administered, the drug must triumph over metabolic and immune processes of the body so that it will remain active for a sufficient time to yield a desired effect without getting degraded and/eliminated. These challenges have motivated and guided the researchers to fabricate new drug carrier/delivery systems to ship and deliver pharmaceuticals in sustained and continuous fashion ensuring the right amount of medicinal agents reaching its desired target imparting desired effects. Generally, drug delivery systems are fabricated biopolymers into which therapeutic agents are loaded (DeStefano et al. 2020).

Transfer of drugs from DDSs to the desired target is controlled by a mechanism which is dictated by the response of the carrier to environmental stimuli such as light, temperature, pH, chemical actions, electric and magnetic fields, etc. (Seddiqi et al. 2021). Biodegradability and possibilities for its manipulation are one or the primary attribute that makes a biopolymer suitable for designing effective DDSs. Degradation kinetics could be prolonged to facilitate and sustain incessant release of medicinal agents enabling the drug to induce its effects. For example, low molecular weight drugs which are highly likely to be immediately filtered and eliminated from the bloodstream through the hepatic and renal systems can be embedded in specific carrier vessels that specifically target specific sites in the body (DeStefano et al. 2020) ensuring optimum therapeutic results.

Wound Healing

Skin, the largest organ in our body, envelops us as a whole and sets up the first line of defense against both physical and chemical threats as a shielding coating. Skin is

often vulnerable to injuries caused by exposure to conditions such as high or temperature, mechanical trauma, microbial attack, contamination, cuts, and so on. Such injuries may cause wounds which might further lead to soreness or such clinical issues ultimately leading to severe secondary injuries if not properly treated in time. Hence skin injuries should be treated quickly and effectively so as to ensure prompt wound healing. A handy and effective way to treat skin injuries is to apply dressing over the wound to facilitate damaged skin repair process. An efficient wound dressing should support the growth and differentiation of injured cells besides setting up material barriers. In general, wound dressing materials should not induce toxic biological responses leading to secondary damages to the affected region. They should also possess adequate gas permeability, capability to absorb the fluids oozing out of the wounds, and should facilitate easy removal. Antimicrobial and anti-inflammatory nature of the raw materials used for preparing such dressings is added advantages. In short, dressing materials should assist the course of curing such as reinstating the defense barrier, averting dehydration, and eliminating the chance for microbial contamination. Further, serious injuries like burn wounds demand skin grafting treatments which necessitate the fabrication of newer bandage materials. In this line, biopolymers have proved their efficacy in wound-dressing applications (Seddiqi et al. 2021).

Key Biomedical Applications of Individual Biopolymers

Cellulose and Starch

In order to use cellulose for tissue engineering applications like primary or scaffold material, it should possess matching mechanical properties to that of real tissues providing porous structures or anchoring sites, for osteoblasts and fibroblasts. The alkyl and ester derivatives of cellulose are mostly used for tissue engineering. Cellulose-based polymers were recognized with high potential for wound-dressing applications (Salihi et al. 2021; Seddiqi et al. 2021).

Hydrogels based on chemically modified and citric acid cross-linked starch are associated with variable hydrophilicity and corresponding variations in swelling characteristics in aqueous environments. These hydrogels can be regarded as environmental friendly and fully biodegradable smart polymeric materials to facilitate controlled release of hydrophilic drugs. The controlled release of levofloxacin a hydrophilic antibiotic drug from modified starch-based hydrogels cross-linked with citric acid could successfully encapsulate and release drugs at physiological pH and temperature (Uliniuc et al. 2013). Similarly, citric acid cross-linked hydrogel films of carboxymethyl cellulose-tamarind gum (CMC-TG) have been found effective for topical drug delivery. Citric acid cross-linked CMC-TG hydrogel films in the form of implants or wound-healing films were investigated for their potential in topical delivery of Moxifloxacin hydrochloride (MH), an extensively employed drug for treating skin sepsis (Mali et al. 2018).

In an attempt to counteract the inherent characteristics like hydrophilicity and poor water resistance which limits the application of starch nanofibers, periodate

oxidation together with adipic acid dihydrazide cross-linking strategy was adopted. The modified cross-linked starch nanofiber showed excellent mechanical strength, superior water resistance, and excellent cyto-compatibility suited for a wide range of biomedical applications (Lv et al. 2020). Physically cross-linked (via freeze–thawing) biodegradable hydrogel membranes made of hydroxyethyl starch, poly vinyl alcohol, and ampicillin were prepared with inherent potential for wound-dressing applications (Kenawy et al. 2014). Bacterial cellulose was improvised using citric acid through thermal cross-linking process preserving its cytocompatibility for wound dressing and scaffolding applications (Salihu et al. 2021).

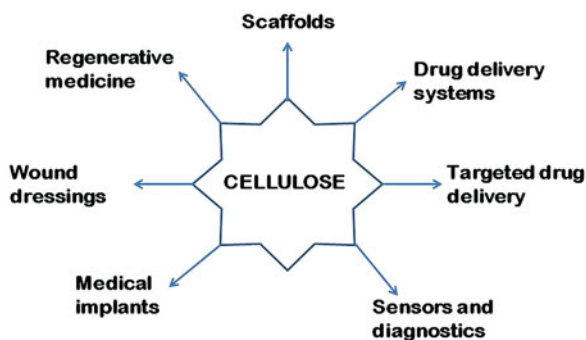
Targeted drug release system delivers pharmaceuticals at predestined body parts through effective manipulation of their reaction to optical, thermal, chemical, electrical, or magnetic stimuli. The inclusion of cellulose and its derivatives in hydrogels changes its structural and morphological properties like enhancement of pore sizes leading to large swelling ratio which in turn promotes stimulus–response rate. Thus, hydrogels for drug delivery can be enhanced in terms of target specificity and biocompatibility through cellulose incorporation (Kabir et al. 2018).

Cellulose nanofibrils have been widely used to prepare hydrogels used to fabricate scaffolds for biomedical applications. But, poor mechanical properties restrict their applications as ink for three-dimensional bioprinting to fabricate scaffolds for tissue engineering. The former issue has been successfully overcome through a dual cross-linking approach in which a hybrid hydro gel ink was produced via Ca^{2+} -mediated cross-linking of poly(ethylene glycol) star polymer and 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO)-oxidized nano-cellulose fibers. The physical cross-links facilitated through interactions between cellulose nano-fibers and calcium ions were reinforced via photo-cross-linking PEG under visible light. This enabled developing a versatile hydro gel ink suitable for 3D printing of bioscaffolds for regenerative medicine applications (Monfared et al. 2021). The major biomedical applications of cellulose are summarized in Fig. 15.

Lignin

Lignin, mostly in a modified form in combination with other materials, has been employed successfully for the production of hydro gels owing to its high mechanical

Fig. 15 Major biomedical applications of cellulose



strength, and inherent antioxidant and antibacterial properties. Lignin hydro gels are highly recommended raw material for scaffolds as they could effectively shield the injured part from secondary further damages or infections. Lignin amine synthesized from sodium lignosulfonate was cross-linked with poly (vinyl alcohol) (PVA) and treated with silver nitrate and was used to fabricate hydrogel scaffolds with enhanced antimicrobial and elastic properties. Another example is the use of lignin obtained from sulfite pulping and chitosan dissolved in PVA to form lignin–chitosan–PVA composite hydrogel. This hydrogel was characterized by a high mechanical strength and high antioxidant activity due to the ionic cross-links between sulfonate groups in the lignin and amino groups in the chitosan. Major physical properties like swelling, hydrophilicity, adsorption, tensile strength, and elongation of the hydrogel were found to increase with lignin concentration. Lignin was found to be a useful candidate for three-dimensional-printed wound dressings. A composite of PLA pellets coated with castor oil, lignin, and tetracycline was used for 3D printing wound dressings with antioxidant and antimicrobial properties. This 3D printing strategy comes along with the underlying potential to realize the concept of customization in the field of wound dressings. Lignin, its derivatives, and composites have also been used for drug delivery applications as they are cheap and cytocompatible. Lignins extracted from sugarcane were successfully fabricated as a methotrexate transporter to treat rheumatoid arthritis (Yu and Kim 2020). The Fe_3O_4 -encapsulated lignin nanoparticle showed super paramagnetic behavior, which makes it promising for targeted drug delivery using magnetic targeting (Spiridon 2018).

Implantation of artificial nerve grafts creates oxidative stresses, resulting in the generation of reactive oxygen species in nerve cells leading to necrosis and DNA damage which can be counteracted by the antioxidant properties of the lignin (Kumar et al. 2021). Hence, the inherent antioxidant property of lignin was employed while fabricating an antioxidative scaffold for nerve regeneration by enhancing polycaprolactone (PCL) nano fibers through preparing PCL/lignin -PCL copolymer composite fibers. Lignin–PCL was found to impart benefits like enhanced technological properties coupled with excellent antioxidant activity and biocompatibility to the fibrous scaffold. Alginate–lignin hybrid aerogels show improved texture, morphology, flexibility, biocompatibility, etc. and showcase wide range of fabrication options in tissue engineering and regenerative medicine (Terzioğlu et al. 2020). The major biomedical applications of lignin are summarized in Fig. 16.

Alginate

The best candidates for injectable scaffolds are hydrogels made from natural proteins and polysaccharides owing to their biocompatible and biodegradable nature and close resemblance with extracellular matrix (ECM). These hydrogels can influence cell resettlement, proliferation, and organization during tissue regeneration to simulate natural wound healing because of their resemblance to ECM. By cross-linking (enzymatic or chemical) or photo-polymerizing their custom-made monomers, in situ gelling biopolymers are created. Alginates possess gelation property in presence of divalent cations owing to the G-moities which are being exploited while using

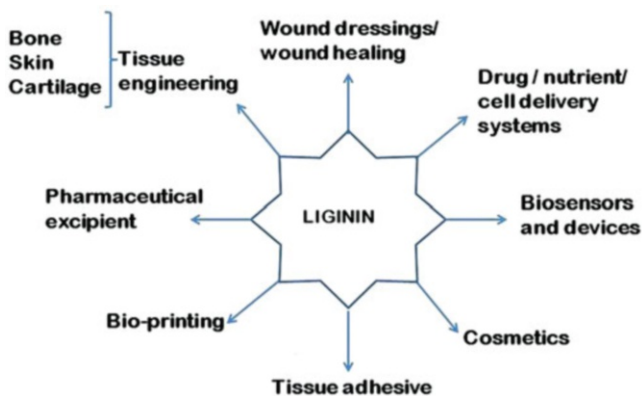


Fig. 16 Major biomedical applications of lignin

them as cell transplantation vehicles and as wound dressings. However, the uncontrollable degradation kinetics and dissolution rates have led to attempts for covalent cross-linking which in turn suffers from the toxicity of chemicals employed. According to reports, oxidized alginate and gelatin can self-cross-link with tiny concentrations of borax to create an injectable, in situ-forming, nontoxic, biodegradable polymer scaffold for use in tissue engineering and medication delivery (Balakrishnan and Jayakrishnan 2005). This is accomplished by placing the cell/drug entity in one syringe, which is in a gelatin solution, and the oxidized alginate in the other, which is in a solution containing borax. The two polymer solutions integrate within the hypodermic needle forming cell/drug entity encapsulated in an insoluble hydrogel matrix through gelation and cross-linking. The primary mechanism for the cross-linking is Schiff's base formation between the alginatedialdehyde and the lysine or hydroxylysine side groups of gelatin. Hydrogels of alginate were fabricated through covalent cross-linking by attaching tetrazine and norbornene groups to it. These hydrogels could efficiently encapsulate and deliver bioactive species in various applications of tissue engineering. Tissue-adhesive hydrogels can assist hemostasis, as sealants, and dressing. An enhanced tissue-adhesive hydrogel has been prepared from gelatin through dual-enzymatic (horseradish peroxidase (HRP) and mushroom tyrosinase) cross-linking system. Through di-phenol interaction between the tyrosine groups on the tissue surface and the phenol groups of gelatin, HRP induced hydrogel-tissue adhesion. Besides, tyrosinase converts phenol moieties into highly activated quinones that can react with various nucleophiles to form a covalent bond which supplements the HRP-induced bonds (Song et al. 2021).

Easy gellation/cross-linking with divalent cations under mild conditions, feasibility of modifying controlled-release performance, and drug loading coupled with provisions for surface modifications/functionalization for targeted delivery makes alginate an ideal candidate for drug delivery applications. Targeted DDSs for cancer therapy envisages enhanced therapeutic efficacy with minimized side effects through their ability to accumulate specifically/preferentially at the tumor site. Several

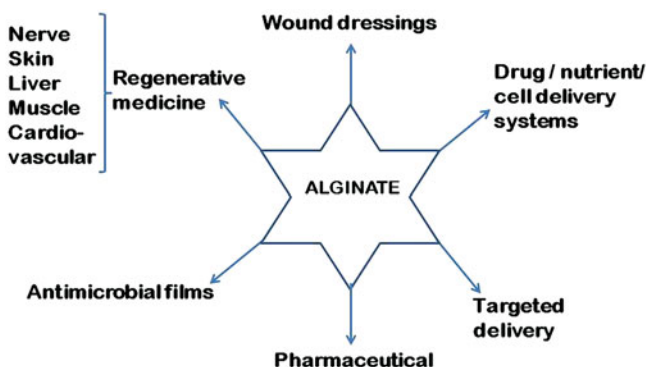


Fig. 17 Major biomedical applications of alginate

nanosized alginate platforms, like nanoparticles, micelles, nano gels, nano droplets, and nano complexes, have been established for targeted DDSs for cancer therapy. Strategies like passive targeting (enhanced cellular uptake, EPR effect) and receptor-based targeting (folic acid receptor-based endocytosis, epidermal growth factor receptor-mediated endocytosis, stimuli-responsive targeting, (pH, ionic strength, temperature, and sensitivity magneto/ultrasound-responsive nature), etc. were extensively used for targeted DDSs for cancer therapy using alginate-based systems (He et al. 2020). The major biomedical applications of alginate are summarized in Fig. 17.

Poly Lactic Acid

A two-phase cell delivery system was created using a mechanically robust elastomeric scaffold and a hydrophilic cell-encapsulating hydrogel for the replacement and/or repair of load-bearing soft tissues via UV cross-linking a lactide-acrylate copolymer and hydrogel of modified chitosan. The scaffolds after UV cross-linking of the elastomers morphology had mechanical properties resembling soft tissues ensuring viable chondrocytes that can effectively proliferate. It was being claimed that such scaffolds are promising alternatives for an injectable and in situ cross-linkable cell delivery system for tissue repair/regeneration (Bednarek et al. 2020). Structural adaptability and biocompatibility make PLA an attractive biomaterial for dental-related uses. Effective osseointegration of dental implants with oral hard tissue is aided by poly lactic acid. A PLA implants have been fabricated to composite materials to hold drugs and assist bone regeneration. PLA is also employed for designing dental resins (with improved mechanical properties) using PLA composite scaffolds which are essential for several restoration procedures. PLA/ Al_2O_3 nano-scaffolds (composite) with increased flexural strength, modulus, and compressive strength compared to traditional resins were synthesized via cross-linking the PLA with Al_2O_3 nano particles (DeStefano et al. 2020).

Biodegradable orthopedic devices are another thrust area for PLA application like resorbable fracture fixation plates. PLA and its copolymers offer increased material

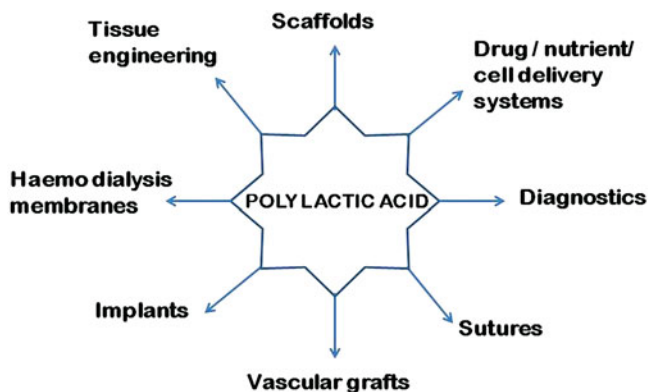


Fig. 18 Major biomedical applications of polylactic acid

strength, prolonged degradation time, robust sterility, etc. enabling effective use in internal bone fixation. Another interesting development is osteogenic or anabolic bioactive infused orthopedic implantables composed of PLA to encourage cell proliferation. Such precisely tailored 3D-printed PLA-based scaffolds with optimized porosity sizes/depth, and connectivity comes along with superior osteo-inductive capabilities. PLA biodegrades to nontoxic products, and it eliminates the need for further surgeries for implant removal. Due to its slow rate of deterioration and ability to withstand dissolving for a long time, PLA has been lauded as a superior drug delivery medium. This characteristic of PLA has been established with cancer drug delivery, wherein compounds like tamoxifen encapsulated in poly (lactic-coglycolic acid) nano particles were found to show enhanced bioavailability in comparison with nonloaded control samples. Additionally, PLA is used in numerous implant techniques to deliver antibiotic agents to sites of implantation which in turn lowers the risks of postoperation infections and probability of implant failure (DeStefano et al. 2020). The major biomedical applications of PLA are summarized in Fig. 18.

Chitin and Chitosan

Chitosan hydro gels are 3D polymer networks. Water infiltration in aqueous environment causes the gel to swell up due to the presence of hydrophilic groups. Cross-linking the gel network through electrostatic or covalent bonds can prevent gel dissolution, thereby increasing the lifetime of the gel and safeguarding its performance. Most of the cross-linkers used for chitosan hydrogel preparation induce an increase in gel toxicity thereby setting a limit on their viability. Natural substance genipin, obtained from *Gardenia jasminoides* fruits, is found to strengthen chitosan hydro gels to improve their mechanical properties through cross-links with remarkably low toxicity. In an environment with a higher pH, genipin can self-polymerize limiting cross-linking efficiency but offers a chance to prepare genipin cross-linked

chitosan with varying properties by varying the reaction conditions to manipulate the extent of cross-linking.

In the recent past, chitosan has been considered as a potential component for Class II hybrid scaffolds with bioglasses. Chitosan is found to be a promising choice for wide range of tissue engineering owing to its peculiar combination of characteristics such as nontoxicity, biocompatibility and biodegradability, and antibacterial properties. Sol-gel synthesis ensured functionalization and covalent cross-linking of chitosan and bioglass with glycidoxypolytrimethoxysilane (GPTMS). Such chitosan-bioglass hybrids are advocated as smart options for regenerative medicine applications with respect to mechanical properties, composition, and structure comparable to human cartilages and bones. Self-cross-linked oxidized alginate/gelatin hydrogels have been developed for cartilage regeneration (Wang et al. 2020).

Due to their biocompatibility and predictable biodegradability, chitosan materials are frequently employed to create guided bone regeneration (GBR) barrier membranes, which limit soft tissue penetration into the graft region during treatments in dentistry that use bone grafts. On the other hand, degradation rates of such membranes may at times be too high for clinical applications. Advances in research have now come up with solutions like cross-linking of chitosan so as to optimize the clinical time frame target of 4–6 months of such membranes. In such an attempt, chitosan mats for guided bone regeneration membranes have been fabricated using genipin to lengthen the degeneration period (Norowski et al. 2012).

Sustained drug delivery is a common application of cross-linked chitosan. A biocompatible hydrogel system for buccal drug delivery was designed such that a catechol functional group was attached to the chitosan backbone followed by genipin cross-linking. A pH-controlled swelling-shrinking nanogel with cross-linked N-lysinal-N'-succinyl chitosan and poly (N-isopropylacrylamide) core enclosed inside a cross-linked bovine serum albumin shell was developed as a potential effective chemotherapeutic drugs carrier. Pharmaceutical and biological applications such as transdermal, parenteral, ophthalmic, and nasal drug administration use chitosan-based nanoparticles, microspheres, hydrogels, films, and fibers. By using the water-in-oil emulsion solvent diffusion technique with ethyl acetate as the oil phase, chitosan microspheres may be created as drug delivery vehicles. AgSD (a combination of silver and sulfadiazine) is a highly recommended drug for topical wound treatment, as it promotes wound convergence and possesses biocidal activities toward a wide spectrum of microorganisms. When the poor water solubility of AgSD was addressed through nanocrystal technology, increased surface area and physical stability after nanosization aggravated the cytotoxicity risks leading to retarded wound healing. Genipin cross-linked chitosan hydrogels were found to be a potential carrier dressing for AgSD nanocrystals promising sustained drug release and reduction of cytotoxicity (de Alvarenga 2011; Gao et al. 2016).

A comparative study of uncross-linked chitosan, chitosan membrane cross-linked with genipin, and chitosan membrane cross-linked with glutaraldehyde was carried out to evaluate the characteristics used as an implantable drug-delivery system. The genipin-cross-linked chitosan membrane was preferred above the other two based on the study's findings for creating an implanted drug delivery system (Mi et al. 2001).

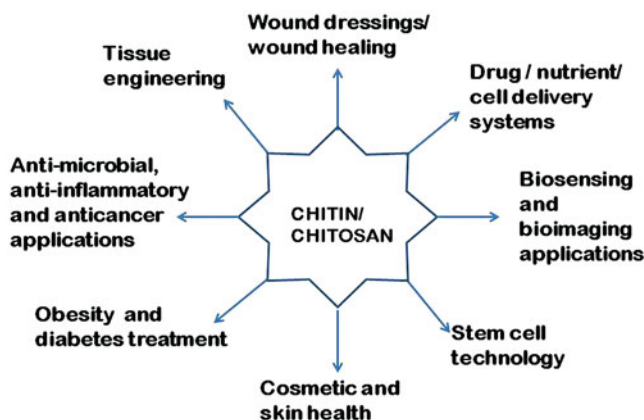


Fig. 19 Major biomedical applications of chitin and chitosan

Though excellent biocompatibility and antibacterial activity favor chitosan to fabricate nerve tubes and scaffolds, pure chitosan cannot be processed due to its high glass transition temperature (T_g of $203\text{ }^{\circ}\text{C}$) and very low thermal stability. One such attempt to employ modified Chitosan was by dissolving it in acetic acid followed by cross-linking with formaldehyde to form porous viscous gel (Mi et al. 2001). This gel was lyophilized and filled with longitudinally aligned poly (glycolic acid) (PGA) fibers and casted into nerve conduits. Physical cross-linking can be achieved by mixing alginate and chitosan/acetic acid solutions owing to their opposite charges which in turn can be used to prepare hydrogel nerve tubes.

The major biomedical applications of chitosan and chitin are summarized in Fig. 19.

Hyaluronan

Hyaluronan (HA or hyaluronate) is a high molecular weight glycosaminoglycan (GAG) found in extracellular matrix of humans. HA possesses a special set of properties like nonimmunogenic, nonadhesive, and bioactive (associated with many cellular processes and axonal in growth) enabling wide prospects for biomedical uses (Angra et al. 2021). HA-based hydrogels, films, scaffolds, foams, topical formulations, and nano formulations are used for wound dressings (Vasconcelos and Cavaco-Paulo 2013). To provide stability and enhance the functionality of gels, HA must first be cross-linked. Chemical cross-linking gives the chance to impart tailored mechanical properties and degradation rates to HA-based hydrogels through side group modification and cross-linking without interfering with its capabilities to perform biological functions. For the transport of molecules and cells, cartilage tissue engineering, and the creation of microdevice systems, cross-linked HA hydrogels are extensively used (Vasconcelos and Cavaco-Paulo 2013; Dovedytis et al. 2020). HA can be cross-linked chemically or physically to form three-dimensional (3D) structures with ample mechanical strength for fabricating nerve conduits. The handling characteristics of cross-linked HA is so weak

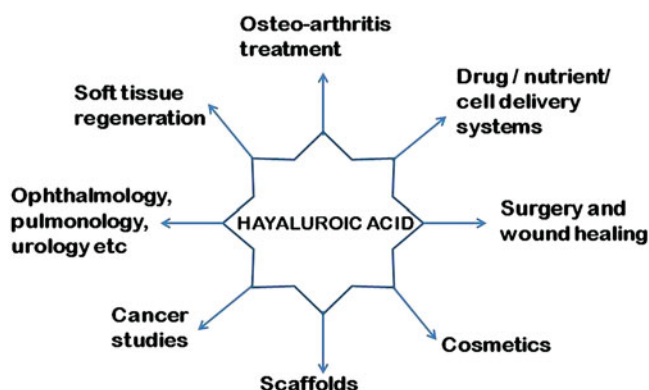


Fig. 20 Major biomedical applications of hyaluronan

that it has to be supplemented by an outer layer prepared from another biodegradable material to ensure its proper functioning during nerve regeneration period (Angra et al. 2021). Because these scaffolds enable greater cell migration and proliferation in wounds, HA nanofibers are thought to be more effective at healing wounds than solid HA. Since HA nanofibers guarantee the quick release of medications when in contact with wet environments, they are also suitable for drug administration. However, due to their fast disintegration and release, they are unsuitable for long-term drug release implants (Vasconcelos and Cavaco-Paulo 2013).

HA scaffolds find role in therapeutic purposes like bone, nerve, and brain tissue repair and muscle regeneration due to their excellent biocompatibility. Large skin defects can also be temporarily covered with HA-based dressings until autologous skin transplants can be employed. Several HA-based products for wound healing are commercially available such as Hyalofill-f, Hyalofill-R, Connettivina, Connettivina Plus, Jaloskin, Hyalomatrix, and Hyalosafe (Vasconcelos and Cavaco-Paulo 2013).

The major biomedical applications of hyaluronan are summarized in Fig. 20.

Collagen and Gelatin

Collagen hydrogels cross-linked with 1,4-butanediol diglycidyl ether (BDDGE) have been found useful for manufacturing temporary dermal scaffolding for explicit skin lesions as they promote tissue regeneration, reduce wound contraction, and reduce immunogenicity. Another example is quercetin-cross-linked collagen a popular temporary scaffold for nonhealing skin ulcers. The initial cytotoxicity invoked by implanted materials leads to chronic inflammation and delayed healing induced by retention of inflammatory cells at the material interface. This often leads to failure and chronic inflammation associated with tissue grafts for complex surgical cases. In this context, cross-linked collagen is found effective in masking immunogenic epitopes, thereby minimizing the chances for host immune responses to trigger off. Researchers have proved that genipin cross-linked collagen scaffolds inhibit proliferation of precise lymphocyte populations and decrease the cytokine release of

helper T cells, all of which contributed toward minimal inflammatory responses (Gu et al. 2018).

A biodegradable cationized gelatin hydrogel has been fabricated to an ophthalmic drug delivery system by air-drying and dehydrothermal cross-linking to deliver an epidermal growth factor. The results of this study suggested that these hydrogels effectively accomplished a controlled release of epidermal growth factor that led to accelerated wound healing (Bhattacharjee and Ahearne 2021).

Enzymatic cross-linking has been employed to fabricate an anti-inflammatory hydrogel for osteoarthritic cartilage repair by Jin et al. in which hyaluronate-tyramine conjugates and gelatin were cross-linked using tyrosinase. In another attempt, collagen-fibril hydrogel was cross-linked by transglutaminase for skin regeneration (Song et al. 2021).

JellaGel is a fibrillar collagen, extracted from jellyfish *Rhizostoma pulmo* formulated to form three-dimensional gels, and is suitable for tissue-engineering applications especially for cartilage restoration with good biocompatibility and little immunogenic. 3D cross-linked JellaGel™ hydrogels fabricated through genipin cross-linking were found to support and facilitate embodiment of human chondrocytes (Riacci et al. 2021).

Gelatin hydrogels modified with methacrylamide (MA) form GelMA, which on exposure to ultraviolet radiation undergoes addition-type polymerization of methacrylamide and methacrylate side groups on the GelMA chains to form a 3D network structure assisted by the presence of a photo-initiator. GelMA hydrogel has been discovered as a promising biomaterial for uses in tissue engineering, including the repair and regeneration of bone, heart, cornea, epidermal tissue, cartilage, vascular, neuron, skeletal muscle, etc. Provisions to manipulate the mechanical properties and the minimum inflammatory response ensured by good biocompatibility make GelMA a reliable candidate for repairing skeletal tissues. GelMA/PEGDA, GelMA/MBG, and GelMA/liposome scaffolds have been developed for bone defect regeneration. Three-dimensional printing technology has been applied to develop multilayered GelMA/HA scaffold for bone defect repair which can repair the damaged bone surface through tissue regeneration and can simultaneously deposit extracellular matrix and type II collagen (Xiang and Cu 2021).

Nerve repair materials fabricated from GelMA hydrogels were further optimized via 3D printing technology and electrospinning technology to ensure better performance. Three-dimensional printing technology has been exploited to improve the efficiency of GelMA/MPEG-PCL [(Methoxy poly (ethylene glycol)-poly (ϵ -caprolactone))] conduits for repairing peripheral nerves. GelMA hydrogel fibers fabricated through electrospinning technology could mimic the components and microstructures of spinal cord in a better way with regard to their good swelling rate and lower Young's modulus and hence effective in spinal cord regeneration.

Unique three dimensional structure and electrostatic interactions facilitate the wide applications of GelMA and its combination with other materials like materials, such as nanomaterials, liposomes, etc. in the drug delivery and controlled drug release field. GelMA for sustained release of abaloparatide for up to 10 days, GelMA-liposome scaffold for controlled release of drugs in stages,

nanocage-laden GelMA hydrogels facilitating pH-assisted controlled drug release, GelMA/chitosan nanoparticles composite hydrogel for promoting cell proliferation and sustained release, and core-shell microparticles fabricated using GelMA for cancer treatment drug delivery are a few instances (Xiang and Cu 2021).

Collagen is a highly favored biopolymer to prepare structures (outer tubular and central lumen) for nerve regeneration. Once, the issues associated with its low mechanical properties are overcome through cross-linking, stable nerve conduits can be fabricated. The three most often used cross-linking reagents are glutaraldehyde, formaldehyde, and the 1-ethyl-3-(3-dimethylaminopropyl)-1carbodiimide (EDC)/N-hydroxysuccinimide (NHS) combination. There is also a cross-linked bovine collagen (type I) nerve conduit tube called NeuraGen (Integra) that has received FDA approval. The potential toxicity of cross-linking agents has led to strategies like microwave cross-linking of collagen with comparable results.

The inconveniences associated with the water solubility of gelatin when circumvented by cross-linking render a suitable biopolymer favoring suitable processing characteristics, mechanical properties, and handling characteristics to fabricate tubular structures for nerve regeneration. Injection-molded or dip-coated gelatin tubes are immersed (the mold or mandrel) into cross-linking agent solutions. Genipin, proanthocyanidin, and EDC/NHS are commonly employed to cross-link gelatin via amino groups on the chain backbone. The free residual amino groups serve as an indicator for determining the cross-link density. A significant factor in determining how quickly a structure deteriorates is the extent of cross-linking, which can also have an impact on how quickly nerves regenerate.

The major biomedical applications of collagen are summarized in Fig. 21.

Keratin

Keratins are naturally derived proteins and are widely used to fabricate structures of several morphologies such as films, sponges, and hydrogels to meet various biomedical requirements in tissue engineering and controlled released applications. The wide range utility of keratin biomaterials is propelled by their inherent advantages such as amino acid sequences that simulate those present in extracellular matrix

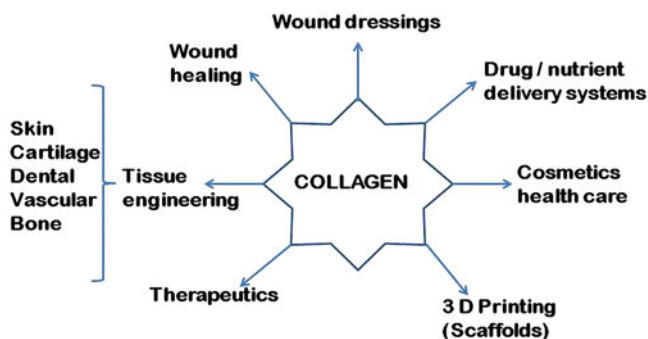


Fig. 21 Major biomedical applications of collagen

(ECM), biocompatibility, and biodegradability. Similarity to ECM facilitates keratins' ability to support cellular attachment, proliferation, and migration (Feroz et al. 2020; Vasconcelos and Cavaco-Paulo 2013).

Keratin biomaterials were successfully employed for controlled drug delivery and bioactive materials delivery. In one such case, diclofenac drug was loaded on keratin films cross-linked with transglutaminase (TGase). It was discovered that the solubility of keratin films was determined by TGase cross-linking which in turn delayed the drug release owing to the enhancement of the mechanical characteristics of the films (Vasconcelos and Cavaco-Paulo 2013). Immobilization of bioactive agents like lysozyme within keratin sponges is facilitated through the presence of free cysteine residues which allows immobilization via disulfide and thioether bonds. The duration period of lysozyme release varied according to the type of cross-linking which has led to the conclusion that judicious selection of the cross-linker, in accordance with the requirements, can guarantee adjustments and regulations over the release characteristics of keratin sponges (Vasconcelos and Cavaco-Paulo 2013). Three-dimensional, biocompatible, biodegradable scaffolds have been fabricated from wool-derived keratin for bone regeneration in which the issues associated with their fragile nature were circumvented through cross-linking keratin fibers. An example for such an approach is the development of a hydroxypropyl methylcellulose (HPMC) cross-linked keratin scaffold for alveolar bone regeneration. This novel scaffold contains hydroxyapatite as a major inorganic component. The major highlight of this attempt is that HPMC is a hydrophilic, biodegradable United States Food and Drug Administration (FDA)-approved polymer which rules out the toxicity issues associated with common chemical cross-linkers. Besides, HPMC offers additional sites for interaction between keratin and hydroxyapatite particles contributing toward highly porous anisotropic structure of the scaffold (Feroz and Dias 2021).

A chitosan-keratin composite possessing enhanced technological properties, degradability, cyto-compatibility, cell adhesion, and growth properties that can cater toward wound-dressing applications was reported to be prepared through UV cross-linking. The fabrication claimed to be more ideal, ensured by the absence of residual toxic effects of chemical cross-linking agents (Lin et al. 2018).

A tenacious and flexible film was prepared from reduced keratin via chemical cross-linking with ethylene glycol diglycidyl ether (EGDE) and glycerol diglycidyl ether (GDE). These chemically cross-linked keratin films were found to be more stretchable and water resistant than keratin–chitosan composite film. Additionally, they kept their mechanical qualities after redrying at par with the original. Though the initial cell attachment on chemically cross-linked keratin was a bit slower, once attached, the cells proliferated well indicating their biocompatibility (Tanabe et al. 2004).

The major biomedical applications of collagen are summarized in Fig. 22.

Albumin

Biomaterials made from albumin rouse increasing interest in the creation of scaffolds for tissue engineering and biomaterials for the controlled delivery of therapeutics

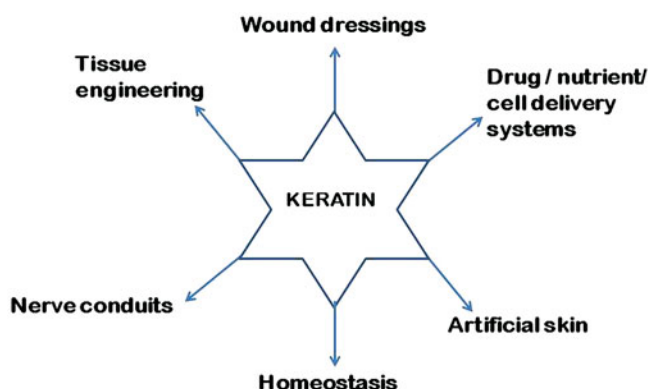


Fig. 22 Major biomedical applications of keratin

such as medicines. Albumin hydrogels inherit nontoxic and nonimmunogenic properties that make them desirable for several biomedical fabrications. Ultraviolet (UV) light-induced cross-linking was used to fabricate bovine serum albumin (BSA) hydrogels with tunable tensile strength and pore size regulated by extends of cross-linking. The 3D scaffolds prepared using these hydrogels were found effective in cell encapsulation and in vivo implantation thereby indicating possible applications in in vitro cancer research, wound-healing techniques, and regenerative medicine (Lantigua et al. 2020). In another approach, poly (ethylene glycol) was used to cross link bovine serum albumin (BSA) through the formation of urethane link between PEG and aminogroups of the protein to form hydrogels that can be used for controlled drug delivery applications and enzyme immobilization (Fortier et al. 1997). A low-cost BSA-based hydrogel was designed through dynamic double cross-linking technique which is expected to realize wide biomedical applications owing to its flexibility, mechanical properties, antibacterial nature, minimal cytotoxic effects, and self-healing capabilities. Glutathione, which joins with BSA to form a disulfide bridge network, and tetrakis (hydroxymethyl) phosphonium sulfate (THPS), which grafts the amino groups of BSA by a Mannich-type reaction, work together to enable the dynamic double cross-linking (Xia et al. 2021).

The effectiveness of small interfering RNA (siRNA) in cancer therapeutics is dictated by its safe and systemic delivery for which several carriers have been widely employed. Human serum albumin (HSA) has been developed as a tumor-targeted siRNA transporter. To increase their binding affinity to one another, thiols were added to both the siRNA and HSA molecules and subjected to chemical cross-linking and self-cross-linking to form stable nano-sized complexes (psi-tHSAs). According to studies, psi-tHSAs aggregated preferentially in tumor locations, where they effectively and sequentially silenced tumor genes exhibiting their possibilities in systemic siRNA therapy of various human cancers (Son et al. 2013).

To create high-performance BSA hydrogels, a hybrid cross-linking technique combining physical and chemical cross-linkings was adopted. Covalently joined BSA molecules via dityrosine bonds were thermally denatured to generate physical

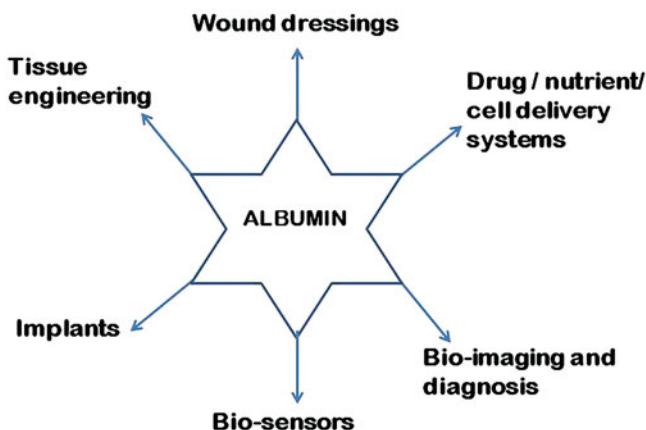


Fig. 23 Major biomedical applications of albumin

cross-links between BSA molecules thereby generating high-strength hybrid dual-cross-links (Lu et al. 2020).

Due to their potential use as tracers and sensors (for molecules, enzymes, temperature, etc.), fluorescent hydrogels are attracting a lot of scientific attention. A biocompatible and biodegradable protein hydrogel with green and red auto-fluorescence was fabricated from glutaraldehyde cross-linked BSA (or HSA) and is likely to elaborate the reach of protein hydrogels in biomedical applications significantly as claimed by the authors (Ma et al. 2016).

The major biomedical applications of albumin are summarized in Fig. 23.

Biomedical Applications of Natural Rubber Latex (NRL)

Natural rubber latex is chemically polyisoprene chains and is comprised of cis 1,4 isoprene units. In natural rubber latex, rubber particles serve as the core of the colloidal system, which is supported by an anionic coating of phospholipids and proteins. Natural rubber latex (NRL) obtained from *Hevea brasiliensis* has been the first and last raw material choice for industrial production of gloves, condoms, balloons, and parts of quite a lot of medical and dental equipments. The protein content present in NRL is believed to be the reason behind latex allergy experienced by some people while using latex products. NRL can be deproteinized through centrifuging and/or with the action of suitable enzymes to obtain deproteinized natural rubber (DPNR) which lowers the risk of latex allergies. Vulcanization is the key process that converts natural rubber from a plastic material to a technologically demanding strong elastic material by a cross-linking reaction. Sulfur vulcanization, radiation vulcanization, and peroxide vulcanization are the three main options for vulcanization of NRL. Sulfur vulcanization creates monosulfide, disulfide, or polysulfide cross-links by the combined action of vulcanization agent (sulfur), accelerator, and activator (zinc oxide and stearic acid) by the application of heat. Both peroxide vulcanization and radiation vulcanization of natural rubber

yields a cross-linked elastomer with simple C–C cross-link structures. The technological properties of sulfur-vulcanized NR are superior to other two methods of cross-linking (Courtney and Gilchrist 1980). The biomedical applications of rubbers can be internal (in vivo) or external (in vitro). Internal uses include both short-term applications such as catheters and long-term implants. External applications include skin contact applications like drug infusion patches and much larger number of medical related applications such as disposable gowns, gloves, sheets, etc. (Mcmillin 2006).

Though NRL has been widely employed for manufacturing medical gloves and catheters, the excellent biocompatibility and ability to stimulate cellular adhesion and extracellular matrix formation promote tissue replacement, and regeneration of NRL has suggested several new biomedical applications such as delivering proteins, drugs, and nanoparticles, enhancing soft tissue curing, and stimulating hard tissue development in guided bone regeneration (GBR) method (Borges et al. 2015). Substitute for arteries, esophagus, pericardium, and abdominal wall have been made using NRL biomembranes. All the experimental results pointed that NRL biomembrane promoted rapid tissue repair through responses resembling normal healing process (Kerche-Silva et al. 2017).

Guided bone regeneration (GBR) technique enhances bone repair especially for large fractures with significant reconstructive problems that demand unusual measures for regeneration. In GBR technique, an occlusive membrane ensures optimum conditions needed for osteogenic cells to perform bone remodeling. NRL membranes when used as occlusive barrier in GBR enhanced bone regeneration process indicated by statistically higher volume of mature bone. The NRL membranes served as a passive protective sheath to stop the migration of connective and epithelial tissue. This made it easier for regenerating bone cells to grow and move into the wound without inducing any foreign body inflammatory reactions (Ereno et al. 2010).

Calcium phosphates (Ca/P) have shown promising applications in the field of orthopedic and dental implants, owing to their inherent structural properties and biocompatibility due to similarity with hydroxyapatite $[\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2]$ a component of bones and teeth. Though Ca/P can promote osteointegration, the brittleness, low mechanical strength, and low biodegradation exhibited by ceramics have to be manipulated for efficient applications. The physiological, chemical, and technical characteristics of NRL plated with calcium phosphate resembled those of the mineral components of bone. Further, hemolytic test results assure that this NRL did not affect the blood cells (Borges et al. 2015).

A dissimilar purpose of NRL in biomedical sense is in the area of magnetic resonance imaging (MRI) which was also investigated. NRL was applied to iron oxide (Fe_3O_4) magnetic nano particles (MNPs) for use as MRI contrast agents. It has been reported that NRL-coated Fe_3O_4 MNPs exhibit improved physical properties proving their effectiveness as a contrast agent in diagnostic techniques. NRL is found to be an angiogenesis-inducing biomaterial and has proved its worth in treatment of diabetic wounds through a number of clinical trials. The wound-healing action of NRL membranes has been found to follow a process in which a Vascular

Endothelial Growth Factor (VEGF)-like material is released from NRL membranes which assist rebuilding veins and arteries and carrying nutrients aiding healing. Hence, NRL has been evaluated as a successful candidate for replacement/regeneration of tissues through several animal trials (Guerra et al. 2021).

Broken bones are treated using complicated and invasive surgery like autografts, allografts, xenografts, the use of synthetic biomaterials, or guided bone regeneration (GBR) techniques. NRL membranes are among the most sensible options for the GBR method since they do not require surgical intervention after healing. By eliminating bone grafts and transplants, NRL membrane offers significant health advantages. Little cost, ease of material availability, and low danger of disease transmission are further benefits. NRL's primary benefits for use as an occlusive membrane include biocompatibility, cell occlusion, osteoconductive, osteoinductive, and osteointegrable nature; vascularization and tissue restoration attributes; constitution and support of the curing area; flexibility, elasticity, and structural strength; and surface porosity and permeability (Guerra et al. 2021).

The drug release kinetics of NRL matrices can be modified by modifying the surface morphology and porous density of the matrix and can be easily tuned for various controlled drug release applications. Moxifloxacin, a broad-spectrum antibiotic, ketoprofen, an anti-inflammatory, ciprofloxacin, a synthetic broad spectrum antibiotic, etc. were successfully subjected to controlled releases upon integration with the NRL. It has been found that 60% of the drug release took place in a controlled manner presenting no cell-damaging effects, with negligible hemolysis level suggesting that the controlled delivery ideally minimizes the adverse side effects associated with systemic drug administration. NRL was suggested as a delivery route for numerous peptides and proteins as well as phyto-constituents with therapeutic qualities because of its benefits for clinical use and cyto-compatibility (Guerra et al. 2021).

Conclusion

Utilizing biopolymers in the biomedical field is an interesting aspect for research as well as the demand of time. Hence, more and more studies are undertaken to optimize and elaborate the use of biopolymers for fabricating biomaterials. In order to suit themselves for biomedical applications, these biopolymers should essentially possess characteristics like low toxicity, biodegradability, biocompatibility, and the potential for further chemical modifications. And most of the biopolymers have these characters embedded in them inherently by nature which act as both a strong pillar of support and a driving force for advanced research while employing them for newer biomedical applications and improving existing applications. Starch, cellulose, lignin, alginate, collagen, gelatin, PLA, HA, chitosan, keratin, albumin, etc. have proved themselves as promising candidates for biomedical applications, either independently, after modification, or in combination with other materials. Research works so far have highlighted the potential of these biopolymers for biomedical application like fabrication of scaffolds, drug delivery systems, tissue-

bone-, and nerve-regenerative medicine, wound healing, etc. However, comprehensive knowledge and advanced understanding of the biological, chemical, and physical properties of these materials is still essential to widen the horizon of their applicability and to fine-tune the existing roles played by them in the biomedical field as most of the researches are in the initial stage presenting in vitro results. Hence it is essential that these promising results are to be extended properly to in vivo studies and to adapt the results with respect to the challenges encountered with different demands in vivo. Emerging research will certainly improve the performance of these materials to assist therapeutic recoveries in order to bring better results to make our lives happier in a healthier fashion.

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Natural Fiber-Reinforced Biopolymers as Construction Materials

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Deepa Narayanan, M. T. Giffin, P. S. Neetha, Cintil Jose, and Sabu Thomas

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D. Narayanan (✉)
Cochin University of Science and Technology (CUSAT), Cochin, India

M. T. Giffin
MG University (MGU), Kottayam, India

P. S. Neetha
CUSAT, Cochin, India

C. Jose
Newman College, Thodupuzha, India

S. Thomas
MGU, Kottayam, India

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Abstract

Building and construction industries are clawing into non-renewable natural resources for scaling up their demands globally. Massive energy expended on the manufacture of raw materials, and giant carbon footprints of construction and demolition have raised environmental concerns. Scientists worldwide are reviving the use of traditional building materials and are striving to develop sustainable eco-friendly raw materials that can be customized to meet demands of building and construction domain. This chapter leads us through one such breakthrough which is biocomposites made of biopolymers incorporated with natural fibers which are durable, strong, sustainable, and eco-friendly. Here we gain an insight into the types of natural fibers used, and how it enhances the properties of biopolymers when developed as biocomposites. Further, it highlights the advantages of biocomposites and several applications. The chapter concludes by addressing the challenges faced in use of natural fiber as reinforcement material and remedies for overcoming these limitations.

Keywords

Biopolymer · Natural fiber · Bio-composite · Construction materials

Introduction

Building material promotion council has estimated that India generates a stark 150 million tons of construction and demolition waste per year. The fact that only 1% of it is recycled and reused is of grave environmental concern. Thus, demanding the development of biodegradable, eco-friendly, and sustainable construction materials. These can be used to replace conventional building materials in constructing transient civil structures like make-shift stadiums, military shelters, shopping malls, and architecture vulnerable to dynamic natural forces, etc. Engineers and scientists take inspiration from age-old traditional construction raw materials and have been developing eco-friendly alternatives. Natural fiber-reinforced biopolymers composites are one of the most promising innovations (Yan et al. 2016). This chapter begins by addressing the need for biodegradable construction materials and emphasizes biopolymers. Further along, it enumerates all the animal and plant fibers currently used as reinforcement material. Finally unfolding the challenges faced in the process, highlighting the outstanding advantages of biopolymer reinforcement with natural fiber, and its innovative and promising applications.

Demand for Biodegradable Construction Material

Environmental issues tagged to the construction industry include massive land space depletion due to landfills, unchecked natural resource consumption, and extreme pollution. The collapse and demolition of abandoned stadiums, tunnels, bridges, and unauthorized buildings, leave behind massive debris which can emit toxic greenhouse gases. Natural disasters across the globe also add millions of tons of debris and have severe socio-economic and environmental impacts. Such crises force us to steer the wheel towards reuse, recycling, and the use of eco-friendly biodegradable construction raw materials.

A biodegradable composite construction raw material should not be mistaken as a vulnerable readily disintegrating material. When compared to nonbiodegradable synthetic construction materials a biodegradable composite with creditable strength and mechanical properties will eventually disintegrate due to microbial action at the site of demolition or collapse but without releasing toxins.

Globally there is an emerging concept of including biodegradable materials in mainstream construction as part of sustainable construction (Sassi 2006). Natural fiber-reinforced biopolymer composites are making a smooth slide into the construction industry owing to their creditable strength, and durability. Fiber composites play a crucial role in rehabilitating bridges and apartments which are exposed to marine salt-corrosive environments. They also find use in the constructions of roofs, girder, floating river walkway pedestrian bridge, water tanks, structural portal frames, and truss systems for deployable shelter (Ticoalu et al. 2010). Use of jute-based composites for key structural applications like interior fundamentals of building, and provisional outdoor purposes like affordable construction for defense, and rehabilitation are increasing. The use of jute as construction materials for developing ceiling panels due to their insulating characteristics is being explored (Sanjay et al. 2016). Straw and coconut leaf sheaths are used for the development of composite building materials (Bharath et al. 2022; Mansour et al. 2007). Load bearing property of bamboo has been an efficient replacement for reinforcing steel in concrete (Sutharsan et al. 2020). However, these are just a few of the many utilities of such materials. There is vast potential for the use of biodegradable materials at a large scale remaining to be uncovered.

Biopolymers in Construction

- (a) Polymers that are extracted from sources of biological origin are commonly referred to as biopolymers. Like all polymers, biopolymers constitute several blocks repeating to form chain-like molecules and can extend to great lengths. Biopolymers are an efficient means for storage and preservation of energy, transmission of genetic information, and cellular construction. They have been regarded as sustainable and eco-friendly as they are biodegradable, thus minimizing carbon footprint. They are obtained either from living organisms or from biological materials which are abundant, renewable, and biodegradable (Shadhin

and Shuvo 2019). Starch-based biopolymers have the potential to replace polyolefins. PLA can substitute Polymethyl methacrylate (PMMA). It is interesting to note that cashew nut shell liquid resin (CNSL) is an emerging biopolymer resin (Lalit et al. 2018).

Some common biopolymers are as follows:

- Polysaccharides such as cellulose, hemicellulose, chitin, chitosan, glucans, and starch
- Proteins such as gelatin, zein, fibroin, and casein
- Derived polypeptides such as gelatin, and collagen peptides
- Polyphenols or modified polyphenols such as tannins, crosslinked tannins, and lignin
- PLA (Polylactide), polyglycolic acid (PGA), poly-b hydroxy alkanates (PHA), polycaprolactone (PCL), and polycaprolactone (PCL)

Applications of biopolymers in the construction industry are listed as follows:

- Use of biogenic polymers as additives or supplements has been reported in construction engineering. One such study shows that treatment of natural soil with beta-glucan enhanced the strength of soil by 300–400% with low CO₂ emission. These qualities displayed by the biopolymer make these ideal choices over. It is also noteworthy that a little amount of biopolymer (about 0.5% of total content) compared to a large amount of cement (10% of total content) can increase the unconfined compression strength of soil (Chang et al. 2015).
- Biopolymers can be used as wood protection agents in construction due to their biocidal action. It reduces the dependence on chemical agents to prevent the destruction of wooden structures (Patachia and Croitoru 2016).
- In the construction and operation of dams, biopolymer slurry is used for drain/reactive barrier trench excavation. It reduces installation costs and time and is much safer. It allows for reduced dewatering so the damage on surround structures is less (Aminpour and O’Kelly 2015).

Natural Fibers: Eco-Friendly Companions of Biopolymer

For a long time, fibers have been used by the construction industry for various purposes. Fiber is the reinforcement used to bear the load transferred through the matrix. They provide strength and stiffness to the polymer (Lalit et al. 2018). Fibers are broadly categorized into two kinds: natural and synthetic (Table 1). Natural fibers are obtained from environmental sources and synthetic fibers are chemically synthesized. Natural fibers are gaining importance because they are greener and more sustainable materials (Mohajerani et al. 2019). Over two decades, the use of fibers as reinforcement materials for polymers has shown positive benefits which include increased strength, lightweight, corrosion resistance, and reduction in the total cost

Table 1 Comparison between synthetic and natural fibers properties (Sanjay et al. 2016)

Aspects	Property	Natural fiber	Synthetic fiber
Technical	Mechanical	Moderate	High
	Moisture sensitivity	High	Low
	Thermal sensitivity	High	Low
Environmental	Production	Low	High
	Resource	Infinite	Limited
	Recyclability	Good	Moderate

of construction. Added advantages are that it takes less labor, less maintenance, and saves time. Unlike traditional reinforcement materials, fibers are required in lesser quantities.

The fiber composites utilize the principle of fibers as reinforcement in the resin matrix. Fiber provides strength and resin provides a binding platform as well as protection for fibers. However, fiber alone cannot sustain actual loads. The type of resin, type of fiber, fiber to resin ratio, industrial procedure, and properties of fiber composites can be selected and combined to develop the desired end product (Ticoalu et al. 2010).

The potential of flax, hemp, and kenaf as composite reinforcement has been studied. These studies have shown that they exhibit properties like specific modulus and strength which resemble the values exhibited by glass fibres (Bari et al. 2021).

Natural fibers are considered to be the new generation of reinforcement materials for polymers and recently, they are seriously being explored owing to their renew-ability, energy conservation applications as well as properties which help them to be nominated as efficient materials in place of synthetic materials (Sanjay et al. 2016).

Natural fibers are easily available and can be obtained from waste materials. The energy required for production is low and also causes less damage to the environ-ment (Mohajerani et al. 2019). Bio-composites are manufactured by using biopoly-mer as a binder and natural fiber as the reinforcement material. For enhancing the strength of bio-composites, reinforcements of natural fibers such as bamboo fiber, ramie, flax, kenaf, jute, etc., are used (Gupta et al. 2019).

Straw-reinforced clay for bricks was the earliest known example of utilization of natural fiber composites (Ticoalu et al. 2010). The use of natural fibers is increasing for constructing low-cost buildings. Structural beams and panels are designed, manufactured, and tested for bio-based composite materials, particularly on plant oil-based resins and natural fibers (Sanjay et al. 2016).

Retting is the process of unraveling and mining the fibers from non-fibrous tissues of plants by detaching, disintegrating, and deteriorating the pectin, gums, and other adhesive substances. Retting can be of varying types based on the method used. This includes biological, mechanical, physical, chemical, or enzymatic retting (Aaliya et al. 2021).

Reinforcing natural fibers to biopolymer matrix requires a fabrication process to prepare preforms and then reinforce the preforms to the matrix. There are several techniques used for fabrication based on the type of natural fiber, biopolymer used,

and conditions for reinforcement. The two main types are open and closed molding techniques. Open molding techniques allow exposure of resin to the atmosphere during curing and include simple unsophisticated techniques which contribute to low processing costs. Some of the open molding techniques are hand lay-up, spray-up, filament winding, etc. Closed molding techniques do not allow exposure of resin to the atmosphere during curing and are opted for constructing three-dimensional composite parts which have better quality, reduced material waste, and aesthetic finished parts. They include techniques like compression molding, injection molding, resin film infusion, etc. (Faruk et al. 2012).

Types of Natural Fibers That Can Be Used with Biopolymers

Natural fibers are broadly classified into two types: animal fibers and plant fibers.

Animal Fibers

Animal fibers are obtained from animal hair, feathers, silk, or any other animal part. Animal fibers exhibit extraordinary chemical, physical, and mechanical properties which make them ideal reinforcement materials. Silk obtained from butterfly larvae is the most potential animal fiber. It shows excellent chemical resistance and mechanical strength owing to the presence of fibroin protein. The tensile strength of silk is approximately 600 MPa. Feather fiber shows good specific strength and is recyclable, making them suitable for reinforcement for developing biocomposites. It was revealed in a study that when a chicken feather along with coir was used as a hybrid, the feather increased the flexural strength whereas coir increased the tensile and impact strength (Prakash et al. 2022). The use of animal fibers in the construction industry is being looked upon and is being explored further. It should also be kept in mind that this also paves way for animal abuse if not regulated strictly.

Plant Fibers

Natural fibers obtained from plants are known as plant fibers and have been used as reinforcement materials for composite development. The chemical composition of plant fibers varies depending upon the plant source. However, they mainly consist of cellulose, hemicellulose, lignin, waxes, etc. Table 2 gives the chemical composition of some plant fibers.

There are four major types of natural plant fibers (Mohajerani et al. 2019) (Table 3)

- (i) **Bast Fibers** – These fibers extracted from exterior parts of plants show high tensile strength. They also showcase good thermal insulation. Examples of bast fibers are flax, hemp, jute, kenaf, etc.
- (ii) **Palm Fibers** – These are the fibers obtained from palm trees. Their mechanical characteristics include low tensile strength, high water absorption, resistance against deterioration, and low modulus of elasticity. Some examples are coir, date palm fibers, oil palm fibers, etc.

Table 2 Chemical composition of some natural fibers (Bari et al. 2021) <http://creativecommons.org/licenses/by/4.0/>

Type of fiber	Cellulose	Lignin	Pentosan	Ash	Silica
Flax	44–57	21–23	24–26	5	–
Jute	57–77	21.26	18.21	0.5–2	–
Kenaf	45–63	15–19	22–23	2–5	–
Sisal	–	7–9	21–24	0.6–1	–
Cotton	–	0.7–1.6	1–3	0.8–2	–
Hemp	87–91	9–13	14–17	0.8	–
Ramie	–	–	5–8	–	–
Bagasse	26–43	19–24	27–32	1.5–5	0.7–3.5

Table 3 Tensile strength and Young's modulus of some natural fibers (Bari et al. 2021)

Type of fibers	Tensile strength (MPa)	Young's modulus (GPa)
Hemp	550–900	70
Jute	400–800	10–30
Flax	88–1500	60–80
Sisal	600–700	38
Coir	220	6
Ramie	500	44
Cotton	400	12
Kenaf	295	–
Banana	355	33.8
Pineapple	170–1672	82

- (iii) **Cereal Straw** – Straw obtained from cereals plants are good natural fibers. They were used throughout history as reinforcement materials. They are good at reducing shrinkage, improving ductility, and enhancing tensile and compressive strength. They also exhibit high water absorption. Some examples are wheat straw, barley straw, etc.
- (iv) **Leaf Fibers** – Leaves of plants contain fibers, fibers which are obtained from leaves and are known as leaf fibers. Some examples are sisal fibers, banana fibers, pineapple fibers, etc. (Figs. 1 and 2).

Plants as a source of natural fibers are elaborated below:

(a) **Jute**

Jute, also known as “Golden fiber,” is a lignocellulosic bast fiber; the scientific name is *Corchorus capsularis* belonging to the Tiliaceae family (Sanjay et al. 2016). They are commonly found in India, China, and Bangladesh as it requires a wet monsoon climate. Jute plants are cut and soaked in water for retting. Jute fiber exhibits reduced thermal conduction, moderate water holding capacity, and good mechanical

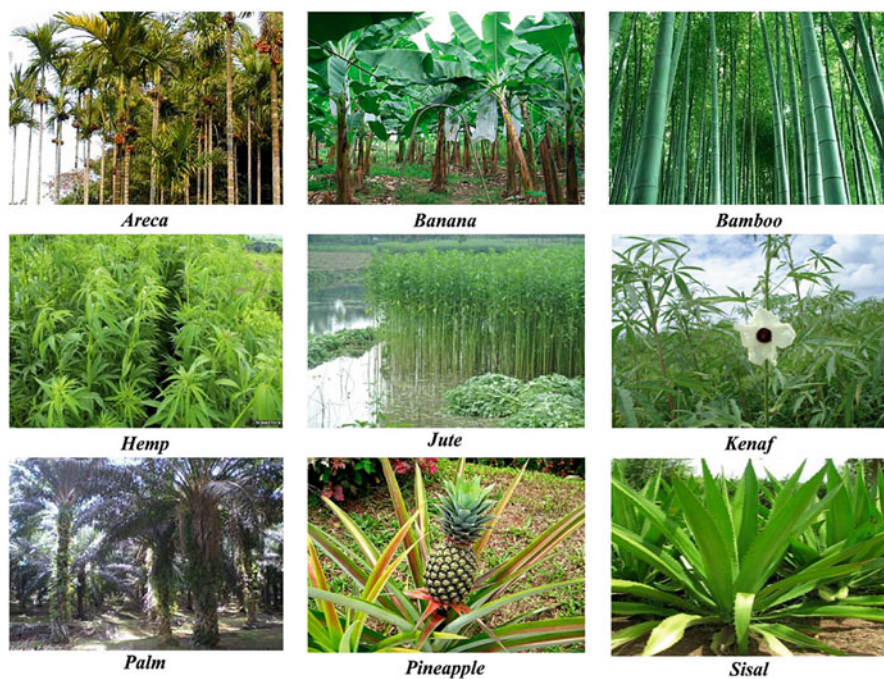


Fig. 1 Plants as a source of natural fiber (Sanjay et al. 2016)



Fig. 2 Examples of biodegradable commercial composites for buildings (Bari et al. 2021)

properties (Aaliya et al. 2021). Jute is the most studied vegetable fiber. They are extracted from the exterior (bark) of jute plants as the fibers are contained in the bark.

They mainly form porous material which helps in filtration, drainage, and soil stabilization and are used in pavement engineering (Mohajerani et al. 2019).

PLA (Polylactide), in combination with jute fabric, increased tensile strength, tensile modulus, flexural strength, impact strength, and water absorption capacity. The bonding between PLA and jute was good and no air voids were present. However flexural modulus was reduced which needed to be investigated further. Jute can be applied in architectural interiors in building construction sector (Gupta et al. 2019).

Another study based on soy protein-jute fiber composite showed that using water as the plasticizer instead of chemicals makes the bio-composite biodegradable and at

the same time useful for various applications because of increased flexural strength, tensile strength, and acoustic properties (Reddy and Yang 2011).

A study by Mohanty et al. showed that surface modification of jute/biopolymer increased tensile strength by 50%, flexural strength by 30%, and impact strength by 90% in composites compared to normal biopolymer (Faruk et al. 2012).

(b) **Flax**

Linum usitatissimum L. is mostly known as flax and is mainly grown for the oil industry but it is interesting to know that it can be used both as reinforcements and as a polymer matrix in bio-composites. The application of flax as reinforcement material is increasing because of its properties which can replace the use of synthetic fibers. The strength and modulus of flax-reinforced composites depend largely on the volume of fiber present. Techniques are being explored for increasing efficient mechanical characteristics of flax fiber (bast fiber) to increase the durability of the flax biocomposites (Shadhin and Shuvo 2019).

(c) **Kenaf**

Kenaf (*Hibiscus cannabinus*, L.) is an herbaceous annual plant. Kenaf and hemp fiber bundles as well as their mixtures significantly increase tensile strength and Young's modulus of composites. They remarkably lower the impact strength of pure Poly lactic acid (Sanjay et al. 2016). Kenaf bast fiber has superior flexural strength with excellent tensile strength which makes it the material of choice for a wide range of extruded, molded, and non-woven products (Babatunde et al. 2015).

(d) **Hemp**

Hemp is an annual plant belonging to the *Cannabis* family. The fiber obtained from hemp is classified as a bast fiber. Hemp fiber reinforced wheat gluten matrix composites were studied by Kunanopparat et al., regarding their thermal treatment and plasticization effect on mechanical properties (Kunanopparat et al. 2008).

Hemp fiber-reinforced Polylactic acid (PLA) fabricated by hot press method was studied for a variation in mechanical properties after alkali treatment on fiber. The results showed that a 40% volume fraction of alkali-treated fiber exhibit the best properties – 54.6 MPa (tensile strength), 8.5 (elastic modulus), and 112.7 MPa (flexural strength) which are much higher compared to PLA alone (Hu and Lim 2007).

(e) **Coir**

Coconut fiber or coir is a lignocellulosic fiber which is present amid the external shell and the husk of coconut (*Cocos nucifera*), a tropical palm belonging to Arecaceae family. Coir shows striking durability and hard-wearing quality (Aaliya et al. 2021).

Coir mainly contains cellulose, hemicellulose, and lignin. The high lignin content makes it more durable compared to other natural fibers. They are used in the

production of building boards, roofing sheets, insulation boards, and building panels, as a lightweight aggregate (Verma et al. 2013).

(f) Sugarcane Straw (SCS)

Sugarcane straw is primarily the waste generated by sugar industry. It is composed of glucans (37%), hemicellulose (28%), lignin (24%), and ash (5%). SCS was studied for its properties to be used as a reinforcement/filler with the biopolymers matrices thermoplastic starch (TPS), Polylactic acid (PLA), and green polyethylene (GreenPE) that are synthesized from renewable sources, and polyhydroxy butyrate (PHB) and polyhydroxy butyrate-co-hydroxy valerate (PHBV) that are synthesized intracellularly from microbial fermentation. The biocomposites were formed by a simple dry blending technique followed by compression molding. The study showed that there was an increase in tensile and flexural modulus. But the water susceptibility was high so these materials are not ideal for humid conditions (Robledo-Ortiz et al. 2021).

(g) Cotton

A study conducted using multi-layer bio-composites consisting of poly(lactic acid) or poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) matrices film stacked with cotton twill fabric showed that among the combinations, cotton- PLA showed better flexural properties. When load-bearing ability, according to standards, was analyzed the results showed good results. This study is promising for the future of bio-composite in construction (Battagazzore et al. 2019). Cotton fibers cause high impact strength but also show lower tensile strength and stiffness (Sanjay et al. 2016).

(h) Sisal

Sisal fiber is a strong, coarse, and hard fiber extracted from the leaves of the sisal plant (*Agave sisalana*) which belongs to the Asparagaceae family (Sanjay et al. 2016). The plant is native to Mexico. It is commercially produced in India, Indonesia, Brazil, Haiti, and East Africa. These fibers show specific strength and modulus comparable to glass fibers and high resistance to insects and pests (Aaliya et al. 2021). Sisal finds application in civil construction, fiber core of steel wire cables of elevators, assortments of building materials, etc. (Bari et al. 2021).

It is seen that when sisal fiber and coir are added in combination to soil blocks, observable cracks are removed, ductility increases and compressive strength also increases (Mohajerani et al. 2019). Sisal- CNSL composites are used for roofing applications (Lalit et al. 2018). According to Zhang et al., sisal/plasticized wood flour composites are fully biodegradable (Faruk et al. 2012; Zhang et al. 2005).

(i) Banana Fiber

Banana fiber is extracted from the stem of the banana plant (*Musa acuminata*) which is a plant native to Southeast Asia. Banana fibers are also obtained from *Musa textilis*

of the family Musaceae. The banana fiber is also known as abaca fiber. Banana fibers display high variability along length and across fibers, a characteristic feature which makes it suitable for applications in natural fiber-reinforced composites. The balance of structure and property shown by banana fiber reinforced composites is the reason which they can be manufactured at a cheaper price (Gupta et al. 2020). Banana fiber is also resistant to seawater.

(j) **Pineapple Leaves**

Pineapple leaf fiber (PALF) is obtained from the leaves of the plant, *Anannus comosus*, belonging to the Bromeliaceae family. The PALF fibers are strong, white, fine, and silky (Sanjay et al. 2016).

Pineapple leaf fiber (PALF) is an excellent candidate for being used as reinforcing filler in green composites due to their superior mechanical properties which are in turn dependent on their high cellulose content. PALF exhibits a rough surface, hence increasing its capacity to be used as an excellent choice for reinforcement with PLA matrix which in turn results in better bonding between PALF and PLA matrix, due to mechanical interlocking. By increasing PALF loading content PALF/PLA composites showed remarkably enhanced tensile properties (Kaewpirom and Worrarat 2014).

Pineapple leaf fiber reinforced polycarbonate composites involve treating the fiber with silane. This results in fibers with the highest tensile and impact strength but thermal stability decreases with increasing fiber content compared to neat polycarbonate. Enhancement of pineapple leaf fiber is carried out by surface modifications like dewaxing, alkali treatment, cyanoethylation, and grafting acrylonitrile onto dewaxed fiber (Faruk et al. 2012).

(k) **Bamboo**

Bamboo (*Bambusa* spp.) is a perennial plant. The fibers are extracted by steam explosion technique. Bamboo fibers show specific strength comparable to glass fibers. Bamboo fiber also shows good absorption of ultraviolet radiation (Faruk et al. 2012).

Mechanical Properties of Natural Fiber Reinforced Biopolymer Composites

The hallmark of using natural fiber-reinforced biopolymer composites for construction purposes is their creditable mechanical properties. These properties are mainly affected by the chemical composition and physical properties of natural fiber and biopolymer, surface modification of the fiber, composite processing techniques (temperature and force applied), processing environment, fiber loading concentration, and the orientation of fiber in the matrix, copolymerization, and plasticization. Waxy substances present in fiber also affect the adhesion and wettability of composites.

The important mechanical properties of biopolymer composites are mentioned below.

Tensile Properties

Tensile strength defines the amount of deformation a material can resist/withstand under the action of external load. Young's modulus (a measure of stiffness) is the ratio between stress and strain of material at the elastic stage. Young's modulus and tensile strength, both depend on the type of fiber and the biopolymer in the biocomposites (Table 4).

Strength and stiffness are more for fiber compared to biopolymers reinforcing fibers to biopolymer matrix enhance the tensile properties of the biocomposites. Tensile properties also depend greatly on the concentration of reinforced fiber.

Flexural Properties

Flexural properties indicate the ability of materials to resist bending deflection when a load is applied to the structure. It is also known as bending strength. Flexural stiffness is one factor which determines deformability. Flexural property is dependent on two factors of material, Young's modulus, and moment of inertia. Hemp, ramie, sisal, and curious fiber-reinforced biocomposites show better flexural modulus of rupture. In some cases, increasing fiber content results in decreased flexural strength due to kinking (which means weak spots in fibers), which causes an increase in the stress concentration in the matrix (Aaliya et al. 2021).

Impact Properties

Impact strength is the ability of a material to resist fracture under stress applied at high speed (Faruk et al. 2012). Generally, natural fibers have low impact strength. The impact strength depends on parameters such as the type of fiber and type of biopolymer of the composite, particle size, interfacial adhesion, etc. The impact strength can be increased by various techniques like treating fibers with silane, etc. (Aaliya et al. 2021).

Table 4 Details about tensile properties of a few natural fibers/biocomposites (Aaliya et al. 2021)

Fibers	Tensile properties
Bast fibers	Highest tensile strength and Young's modulus
Flax	High tensile strength and Young's modulus due to low microfibrillar angle and high cellulose content
Ramie and curaua	Excellent rupture modulus
Hemp	Stiffness greater than E-glass fibers
Ramie fiber (unidirectionally oriented)-reinforced soy protein concentrate	Tensile properties are comparable to steel
Coir	Low tensile strength due to high microfibrillar angle and low cellulose content

Dynamic Mechanical Thermal Properties

DMTA (Dynamic Mechanical Thermal Analysis) is used to determine the Heat Deflection Temperature (HDT) of biopolymer composite. The elastic response (storage modulus), viscous response (loss modulus), and damping ($\tan \delta$) are values of material given as functions of temperature at 3 °C/min. A strong modulus for a natural fiber-reinforced biopolymer is good. A study by Oksman et al. demonstrated that flax reinforced PLA composite shows increased strong modulus (Oksman 2001; Aaliya et al. 2021).

Toughness and Hardness Properties

The strength and ductility of a biopolymer composite determine its toughness and hardness. Toughness is defined as the ability to absorb energy and plastically deform without fracturing is regarded as the toughness of a material. Hardness is the ability to resist permanent plastic deformation when a compression force is applied (Aaliya et al. 2021).

Creep and Fatigue Properties

Creep in a biopolymer composite is the tendency to deform slowly but permanently due to mechanical stress. Fatigue is the structural damage due to cyclic loading and unloading of material. It depends on the shape and structure of the biopolymer composite (Table 5).

Brittleness and Ductility Properties

If a biopolymer composite under stress, breaks with or without noticeable deformation then the material could be considered brittle or ductile. Natural fibers deform before fracture compared to glass fibers. Various studies show that natural fiber reinforced biopolymers have enhanced ductility due to small-sized fiber particles, surface area, and elevated aspect ratio of the fiber. Homogeneous distribution of fiber in the matrix and strong interaction between fiber and matrix also contribute significantly (Aaliya et al. 2021).

Tribological Properties

Tribology is the study of friction and wear of two mating surfaces. Research reveals reinforcing natural fibers on biopolymer matrix, friction, and wears performance can be improved (Aaliya et al. 2021).

Table 5 Mechanical properties of various natural fiber reinforced composites (NFRCs) (Syduzzaman et al. 2020)

Reinforcement fiber	Matrix	Treatment (fiber/matrix modification)	Fabrication technique	Properties		
				Tensile strength (MPa) and modulus (GPa)	Flexural strength (MPa) and modulus (GPa)	Impact strength (KJ/m ²)
Hemp	Polylactic acid	Alkali treatment (4 wt.% NaOH)	Compression molding	6%↑-strength 17%↑-modulus	8%↑-strength 14%↑-modulus	17.5%↑
Sisal (20 wt.%)	Glycerol/thermoplastic starch	Compared to neat thermoplastic starch	Roll mill mixing and hot-press molding method	115%↑-Strength 1410%↑-modulus	–	–
Jute fiber	Polylactic acid	Compared to jute/polypropylene	Hot-press technique	52.6%↑-strength 119%↑-modulus	130.8%↑-modulus	–

Advantages of Natural Fibers as Reinforcement Materials of Biopolymer

Biopolymers and natural fiber composites help in reducing overreliance on non-renewable energy/material resources. They decrease greenhouse gas emissions and pollutant release, improve energy recovery, and increase biodegradability. These sustainable materials lower the structural cost and weight. It increases structural performance (Battegazzore et al. 2019; Yan et al. 2016).

Natural fibers (jute, sisal, pineapple, banana, flax, and hemp) because of their exceptional properties such as reduced density, biodegradable nature, non-corrosive nature, vast accessibility, non-toxic nature, and low carbon release can be suitable candidates for replacing the synthetic fibers. The alluring highlights of the natural fibers are their ease of procurement, high specific modulus and light weightiness (Prakash et al. 2022).

The use of natural fibers as reinforcement of polymer and cement matrices improves the mechanical properties of the matrices such as tensile, flexural, and impact properties, etc. (Yan et al. 2016). The reinforcing property of the natural fibers is due to their lignin content and their crystallinity (Gupta et al. 2020).

Thermal applications find the use of natural materials beneficial since they show significant thermal insulation properties and can regulate temperature and humidity with more ease. (Mohajerani et al. 2019). Fiber composites also have properties like high strength, lightweight, water resistance, chemical resistance, fire resistance, and resistance to corrosion. The properties can be engineered according to needs and have proven to be cost-effective (Ticoalu et al. 2010). Laminations and panels made from a combination of polyester, phenolic, or polyurethane resins with sisal, jute, and coir fibers show unique properties similar to their synthetic alternatives (Bari et al. 2021).

Challenges of Using Natural Fiber Reinforced Biopolymer Composites

Albeit biopolymers are abundant and eco-friendly, there are certain challenges which have to be addressed before large-scale application. Biopolymers do not possess all the desired properties which are characteristic of synthetic polymers. They have limited mechanical properties. Hence, manufacturing biopolymers of desired shapes and sizes can be difficult at times. Biopolymers also degrade much faster; so, the development of the same should have predictable degradation pathways. Sometimes the current pieces of equipment cannot be used to process the biopolymers properly and therefore overcoming these limitations include the use of natural fibers to reinforce the biopolymer matrices. This improves the properties whilst maintaining biodegradability (Bari et al. 2021).

Factors such as the hydrophilic nature of plant-based natural fibers and hydrophobicity of the polymer in biocomposites may result in poor interfacial bonding between matrix and reinforcing material. Other factors which are a hindrance in the development of natural fiber reinforced biopolymer are mainly low thermal stability,

high moisture absorbency, and low wettability of cellulose content. Moreover, the properties of natural fibers vary depending on changes in weather, season, cultivation conditions, and processing techniques (Syduzzaman et al. 2020).

Another challenge of using natural reinforced materials is difficulty in gauging durability (Aaliya et al. 2021). Biocomposites have negative impacts on them due to weathering factors such as humidity, temperature, rain, and UV radiation.

Factors which govern the durability of biocomposites are:

Moisture Absorption

Both natural fibers, as well as biopolymers, are plasticized by water. Hence it reduces the stiffness of composite resulting in higher moisture absorption which indicates loss of tensile and flexural properties and isn't considered an ideal indicator. The fact that natural fibers have high moisture absorption capacity is another challenge.

Thermal Stability

Another concern amongst biopolymers is low thermal stability. However, reinforcing with natural fiber enhances the thermal stability of biopolymers but care must be taken for getting the desired properties of composites by the adequate and appropriate blending of the fiber and matrix which have different thermal properties.

Fire/Flame Retardancy

When natural fiber-reinforced biopolymers are used for building materials, flammability and flame retardancy property are of prime concern as natural materials are easily flammable. Biopolymer composites show varied flammability depending upon the parameters which control them which include; the type of natural fiber and biopolymer, type of reinforcement, adhesion between reinforcement and matrix, and the structure of the composite. Various additives are mixed with biopolymer composites to improve their flame resistance without affecting their mechanical properties. An example is kenaf/E-PHBV composites which when incorporated with metal oxide (Sb_2O_3) and a phosphate-based additive, act as a flame retardant. But there are major limitations to adding certain flame retardants like halloysite nanotube as it weakens the mechanical properties. Therefore, more research has to be carried out to determine new fire retardants which do not lessen the mechanical properties.

Ultraviolet Resistance

Lignin which is present in natural fibers is susceptible to photodegradation which imparts a yellow discoloration to the composites. UV radiation reduces covalent

bonds in biopolymer resulting in surface roughening, degradation of mechanical properties, and embrittlement that are harmful to the composite. An experiment done by Campos et al. showed that UV exposure on thermoplastic like sisal/starch composite reduced their mechanical properties and produced holes in the surface, causing embrittlement and discoloration. The addition of chemicals to strengthen the cell wall polymers has shown reduced lignin degradation and also has been key in keeping degraded fibers structures together on UV exposure. Yet, the use and availability of such chemicals without compromising on the mechanical properties and sustainability are still in the pipeline.

Biodegradability

Biodegradation is the ability of the material to serve as a platform for microbial growth. Natural fiber-reinforced biopolymer composites derived from PHB, PBS, and PLA, are biodegraded by anaerobic digestion and/or enzymatic degradation. A study by Barkoula et al. showed that flax fiber-reinforced PHB matrix composite had reduced tensile strength in the primary stage of biodegradation studies. Although the presence of lignin reduces the microbial attack, all-natural fibers are not lignified. Biodegradability can be reduced by making natural fibers less hygroscopic as well as less susceptible to enzyme degradation. But if chemical agents are to be added then the whole purpose of the eco-friendly approach will need to be considered from square one. Therefore, the field faces major challenges like these which need more exploratory research that focuses on finding ways to make durable bio composites without compromising on mechanical properties and sustainability principles.

Overcoming the Limitations of Natural Fiber as a Biopolymer Reinforcing Material

One of the major disadvantages of natural fibers is high moisture sensitivity (Sanjay et al. 2016).

They have the drawback of poor durability due to their natural origin so degrade with time and plant fibers are hydrophilic which are being studied to enhance the overall efficiency of natural fibers (Mohajerani et al. 2019).

When a mechanical property is considered on a whole it is found somewhat inferior to synthetic fibers but works are in progress to improve these problems of mechanical properties. The compatibility of biopolymer and its corresponding natural fiber can suffer greatly due to the hydrophilic nature of the natural fiber. Certain chemical and physical methods are under experiment to overcome the barrier (Lalit et al. 2018).

Before processing reinforcement fibers, they must be modified to improve the interfacial bonding usually by improving the wettability of fibers and moisture absorption of fibers/matrix. Interfacial bonding between the reinforcement fibers

and the polymer matrix is improved by implying fiber modification techniques which can be chemical or physical methods (Syduzzaman et al. 2020).

When chemical treatment is done for surface modification of fibers it reduces the hydrophilic nature and increases the fiber-matrix interaction thereby improving the tensile properties of biocomposite (Aaliya et al. 2021).

Physical modifications of natural fibers include methods like stretching, calendaring, thermo treatment, and the production of hybrid yarns. These modifications help change structural and surface properties for improving mechanical bonding while keeping chemical composition intact. The interface is enhanced by an increase in mechanical bonding only (Faruk et al. 2012) (Table 6).

Chemical modifications of natural fibers are mainly done using chemicals like alkali, silane, acetyl, etc. In a chemical modification, the chemical used causes changes in the hydrophilicity of natural fibers by altering the hydrogen bonding, etc., and thereby increasing interfacial adhesion and other mechanical properties (Table 7).

Some of the future trends that should be focused to enhance natural fiber reinforced biopolymer development and their importance in the market are (Aaliya et al. 2021):

Standardized protocol development for composite preparation which will help in proper time management, energy and raw material optimization.

Developing optimized natural fibers by modification techniques for ensuring quality.

Novel investigational systems have to be designed to monitor and control factors like durability and biodegradability of natural fiber reinforced biopolymer composites.

Table 6 Physical modifications on some fiber and their improved property (Faruk et al. 2012)

Fiber	Physical modification method	Property improved
Hemp, flax	Corona treatment	Increased tensile strength and modulus
Flax	Plasma treatment	Increased stiffness
Jute	Plasma treatment	Increased interfacial adhesion

Table 7 Chemical modifications on some fiber and their improved property (Faruk et al. 2012)

Fiber	Chemical treatment	Property improved
Kenaf	Silane treatment	Increased strong modulus, reduced $\tan \delta$
Coir	Silane treatment	Increased tensile strength, modulus, hardness, etc.
Sisal	Alkali treatment	Improved compressive strength and water resistance and interfacial adhesion
Pineapple leaf	Alkali treatment	Improved mechanical properties
Ramie	Alkali treatment	Improved tensile strength
Jute	Alkali treatment	Improved flexural strength
Flax	Acetylation	Reduced moisture absorption
Abaca	Acetylation	Improved tensile strength, modulus, and impact strength

Structural applications for the construction industry need the development of load-bearing, long-term natural fiber reinforced biopolymer composite with remarkable strength and properties.

The reusability of composites is to be explored further as they can be used once again after proper processing.

The development of nanofibers from natural fibers for biocomposites should be explored for constructions as nanotechnology-based coatings help to increase water uptake, reduce biodegradation and volatile organic compounds and even flame resistance. They can improve performance, durability, value, service life, and utility while at the same time ensuring the quality of the material.

Conclusion

Millions of tons of waste generated by the construction sector is one of the prime reasons for environmental degradation. Land space is being consumed and landfills are getting depleted, water and air are being contaminated, and natural resources are overexploited. Globally there is an escalating demand for modern infrastructure and architecture that keeps this sector sourcing humungous quantities of construction raw materials.

Taking responsibility for their negative role in ecological disruption, engineers have resorted to recycling, or direct reuse of materials through integration into construction or as fill dirt.

The problem still is prominent and has to be dealt with at a different level which is biodegradable alternative construction material.

Natural fiber-reinforced biopolymer composite combines the advantages of both the materials and gives rise to a stronger alternative for construction. The biopolymer serves as the matrix for the reinforcement of natural fibers. The mainly used biopolymers for the purpose are PLA, Thermoplastic starch, CNSL, etc. Natural fiber may be of animal or plant origin, majority of research is done on plant fibers as they are available abundantly, and have less production cost, with comparable properties to the synthetic ones. Animal fiber is new to the list and has excellent properties needed for construction. Bird feathers, silk, etc., are commonly used. Silk shows excellent properties like tensile modulus as that steel. Hence, more research is to be done on animal fibers. Some of the products developed using these bio composites are roofing materials (e.g., Sisal-CNSL composites), building boards (coir composites), panels, doors, etc.

The topic is of great importance for the biopolymer sector, as the construction industry will meet its needs using biopolymer reinforced with natural fiber as they offer ease of availability, biodegradability, and strength comparable to synthetic counterparts and due to ever-increasing reliance of the construction industry on raw materials.

Natural fiber reinforced biopolymer possesses some limitations like durability, require treatment for increasing interfacial adhesion between natural fiber

(hydrophilic) and biopolymers (hydrophobic), photosensitivity against ultraviolet radiations and less flame resistance.

Future research is focused on developing techniques which can overcome the limitation and challenges impeding the advancement at present in the field. Developing natural fiber hybrids which can be reinforced with biopolymers for fabricating composites with supreme characteristics. Nanofibers from natural fibers have to be explored since they have comparable strength as that to steel and aluminum. So reinforcing nanofibers along with biopolymer will be more effective and feasible in future.

The future of the construction industry is going to be sustainable with the development of such alternative building materials.

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Polylactic Acid (PLA)

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Applications

Anju Paul, K. Sreedevi, Sreekala S. Sharma, and V. N. Anjana

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Abstract

Polylactic acid (PLA) is an excellent biopolymer that can be synthesized from renewable resources. It is a thermoplastic polyester generated from starch, rice, and corn. The features of PLA such as renewable origin, easiness in composite formation, simplicity in processing, and recyclability make it an exceptional polymer in many commercial applications. Its biodegradability, biocompatibility, and bioabsorbability extend its interest in biomedicine. These properties also make PLA stand out as a good choice when environmental pollution is

A. Paul (✉) · S. S. Sharma

Department of Chemistry, Sree Sankara Vidyapeetom College, Perumbavoor, India

K. Sreedevi · V. N. Anjana

Department of Chemistry, Sree Sankara Vidyapeetom College, Valayanchirangara, Kerala, India

concerned. These features assist the material in substituting conventional polymers in many fields of applications. The mechanical, thermal, and rheological properties of PLA create contributions to medical, food packaging, textile, and many other industries. Blending PLA with other biopolymers results in value-added composites that have features better than neat PLA. The chapter focuses on various properties of PLA and the latest developments in its applications in different fields.

Keywords

Polylactic acid · Biopolymers · Renewable resources · Biomedical applications · Green packaging · Textile applications · Automotive applications

Introduction

Traditional fossil-based plastics like polyvinyl chloride, polyethylene terephthalate, and polyethylene which have become an ubiquitous part of our daily lives are nonbiodegradable and persist in the environment for a long period of time, adding to the exponentially increasing carbon footprint (Agrawal 2010; Malinconico et al. 2018; Zheng and Suh 2019). The increasing demand for energy and decline of petroleum-based resources has forced researchers to look for bio-based, greener, sustainable, and more economic alternatives. Bio-based polymers are the front-runners in replacing petroleum-based ones which are marred by their severe economic, health, and environmental impacts (Chen and Yan 2020; Pellis et al. 2021; Sousa and Silvestre 2022).

Biopolymers are derived from natural resources. They are either biosynthesized entirely by living organisms or chemically synthesized from biological materials. A majority of the biopolymers can be degraded into nontoxic small molecules like water and carbon dioxide with the aid of microorganisms, making them an environment-friendly material (Aaliya et al. 2021; Baranwal et al. 2022; MacGregor 2003). Currently, bioplastics represent only a small-scale market compared with conventional plastics. However, the global market for bio-based polymers is expected to undergo significant growth in the coming years as a result of the increasing demand for greener materials and stringent regulatory policies on the use of nondegradable synthetic polymers (Masutani and Kimura 2017; Rodríguez et al. 2020; Song et al. 2018).

Among the biopolymers, PLA is touted to be the most promising and economically viable polymer and has received extensive attention from researchers in the past few decades. Attractive properties including biodegradability, biocompatibility, nontoxicity, thermoplasticity, high strength, high modulus, and good processability has made this polymer an ideal candidate in many areas of applications like biomedical devices, food packaging, disposable household items, and agricultural films (Ahmed and Varshney 2011; Lasprilla et al. 2012; Jandas et al. 2013a; Calcagnile et al. 2019; Liu et al. 2020a; Knoch et al. 2020; Boey et al. 2021). Innovations and developments in processing techniques of high molecular weight

polymers have led to the large-scale industrial production of PLA, which has brought down its cost to a great extent (Kühnert et al. 2018). An abundance of literature is available on the synthesis, properties, and applications of PLA. This chapter aims to present these results in a concise manner, with main focus on the applications of PLA in diverse fields.

Synthesis of Polylactic Acid

Poly(lactic acid) or polylactide (PLA) is a linear aliphatic thermoplastic polyester of lactic acid, which can be easily obtained by fermentation of renewable carbohydrate resources. Glucose, sucrose, lactose, maltose, and starch are the commonly utilized carbohydrates in lactic acid fermentation and are produced from feedstocks such as beet sugar, molasses, whey, and barley malt. Microbial fermentation is a frequently employed approach for the preparation of lactic acid. Fermentation temperatures vary, depending on the microorganisms utilized, but are normally between 30 and 60 °C with a pH of 5.0–6.5 (Masutani and Kimura 2018; Montané et al. 2020; Pretula et al. 2016).

The monomer, lactic acid, exists in two optically active configurations, D-lactic acid and L-lactic acid. Hence, PLA has several configurational isomers in which the D- and L-lactic acid units are present in different ratios and different sequences. Poly(L-lactic acid) (PLLA) and poly(D-lactic acid) (PDLA) are enantiomeric polymers that solely include L- and D-units, respectively, whereas poly(DL-lactic acid) (PDLLA) is a racemic polymer that contains a random sequence of both enantiomeric units (Fig. 1). Different grades of PLA with a wide range of properties can be prepared by varying the ratio of the D- and L-isomers (Averous 2008; Chan et al. 2018; Dorgan et al. 2000; Gupta et al. 2007; Rivero et al. 2017).

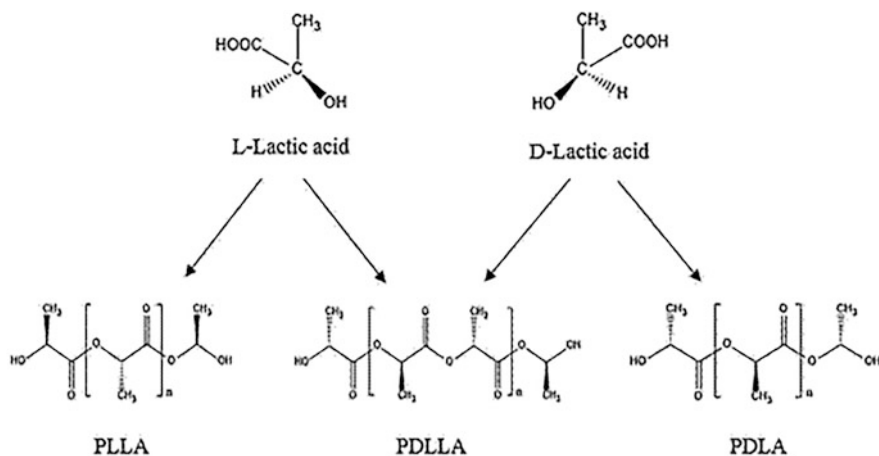


Fig. 1 Stereoisomeric forms of lactic acid and poly(lactic acid) – poly(L-lactic acid) (PLLA), poly(D-, L-lactic acid) (PDLLA), and poly(D-lactic acid) (PDLA). (Reproduced from Rivero et al. 2017. Copyright © 2017 with permission from Elsevier)

PLA was first synthesized by Theophile-Jules Pelouze in 1845 by condensation of lactic acid (Blukis 1992). Later, Wallace Hume Carothers et al. devised a technique for polymerizing lactide to generate PLA in 1932, which DuPont patented in 1954 (Carothers et al. 1932; Lunt 1998). PLA is currently approved for use in food and medical applications by the US Food and Drug Administration (FDA) and European regulatory authorities (Li et al. 2020; Singhvi et al. 2019).

PLAs can be synthesized by three different routes, viz., direct condensation polymerization, azeotropic dehydration condensation, and ring-opening polymerization (ROP) of lactide, as shown in Fig. 2 (Li et al. 2020; Masutani and Kimura 2018; Rivero et al. 2017).

Direct polycondensation of lactic acid is a two-step process in which first lactic acid undergoes self-esterification through a reversible step-growth mechanism to form low molecular weight oligomers (or prepolymers), which in a second step will form higher molecular weight polymers with the aid of chain-coupling agents (Balla et al. 2021; Masutani and Kimura 2014). Both these steps are reversible and require the removal of water, which is formed as a side-product, to shift the chemical equilibrium in the forward direction. The water removal which is a crucial step in this reaction becomes difficult with increase in the molecular weight and hence viscosity of the polymer. Hence, this method is more suitable for the preparation of low molecular weight PLA (Montané et al. 2020; Ren 2011; Södergård and Stolt 2010).

Azeotropic dehydration condensation is a modification of the direct polycondensation method wherein an azeotropic solution is used to increase the efficiency of water removal. This method was patented by Mitsui Toatsu Chemicals in 1994. In this method an organic solvent with a high boiling point is used as the azeotropic solvent which forces the removal of water and shifts the equilibrium in the direction of esterification. This is a single step process in which the equilibrium between the

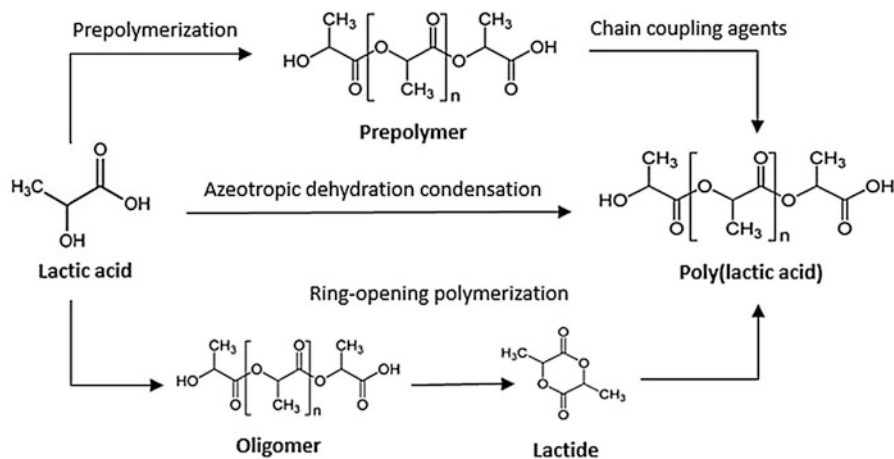


Fig. 2 Synthesis of poly(lactic acid). (Reproduced from Rivero et al. 2017. Copyright © 2017 with permission from Elsevier)

monomer and polymer can be tuned by choosing appropriate organic solvent. PLA with comparatively high molecular weight can be synthesized by this method. The solvent selected in this method plays a key role in the polymerization conditions and properties of the final PLA product (Montané et al. 2020; Sabu et al. 2014; Södergård and Stolt 2010).

Ring-opening polymerization of cyclic lactide diesters is the most popular method used for the preparation of high molecular weight PLA on an industrial scale. As mentioned before this method was first reported in 1932 by W. H. Carothers. In this technique lactic acid is converted into low molecular weight prepolymer by condensation reaction, which in turn is converted into its cyclic dimer or lactide by controlled de-polymerization. The lactide after purification is allowed to undergo ring-opening polymerization to yield PLA with controlled molecular weight (Carothers et al. 1932; Montané et al. 2020; Södergård and Stolt 2010; Thongchul 2013). Different catalysts based on Al, Zn, Sn, Mg, Ca, Fe, Ti, Sm, Y, Lu, and Zr have been used for ring-opening polymerization, of which stannous octoate is the most extensively studied one because of its high catalytic efficiency (Ghalia and Dahman 2017; Jiménez et al. 2014; Stefaniak and Masek 2021; Toshikj et al. 2020). Ring-opening polymerization of the lactide can take place through three different mechanisms: cationic, anionic, and coordination insertion mechanism. The type of mechanism followed depends on the catalyst used (Masutani and Kimura 2014; Mehta et al. 2005; Stefaniak and Masek 2021). Complete discussion on the different ring-opening polymerization mechanisms is beyond the scope of this chapter and can be found in various reviews and books dedicated to synthesis of PLA (Masutani and Kimura 2018; Mehta et al. 2005; Södergård and Stolt 2010; Yao and Yang 2009).

Properties of Polylactic Acids

Thermal Properties

The degree of crystallinity determines many of the properties of PLA (Gawel and Kuciel 2020; Jiménez et al. 2014). PLA is a semicrystalline polymer with a glass transition temperature (T_g) of around 50 °C–80 °C and a melting temperature (T_m) of about 130 °C–180 °C (Ren 2011; Vouyiouka and Papaspyrides 2012). Different structural characteristics, such as molecular weights and composition (percentage of stereoisomers), could influence PLA's thermal performance (J Ahmed et al. 2009; Hamad et al. 2015). The impact of molecular weights and composition (L/D ratio) on the thermal characteristics of PLA polymers was studied by Dorgan et al. (Dorgan et al. 2000). They showed that the T_g value of PLAs varied with the number average molecular weight according to the Flory-Fox equation:

$$T_g = T^\infty - \frac{K}{M} \quad (1)$$

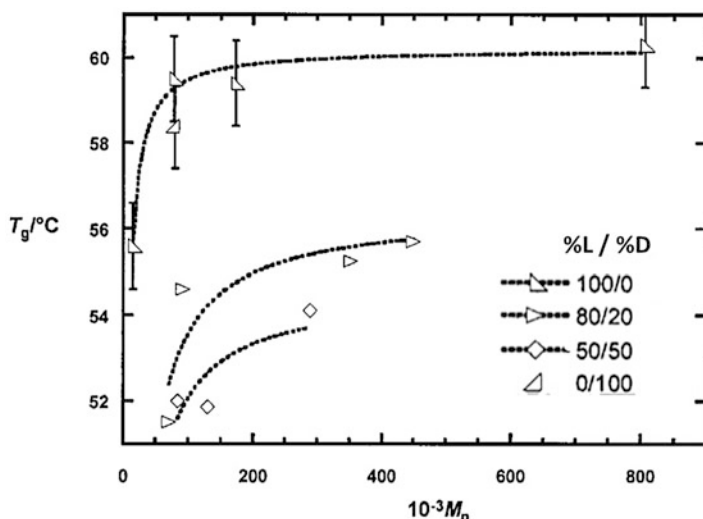


Fig. 3 Glass transition temperature of PLAs with different L/D ratios as a function of number-average molecular weights (M_n). (Reproduced from Dorgan et al. 1999. Copyright © 2005 with permission from AIP Publishing)

where T^∞ is the glass transition temperature at infinite molecular weight, M is the molecular weight, and K is a constant and is related to the free volume of the end groups for the polymer chains. Figure 3 shows the variation of glass transition temperature with change in composition and number-average molecular weight of PLA.

Several studies have shown that the crystallinity of PLA is determined by its optical purity. Differential scanning calorimetry is the most common method used for determining the crystallinity of PLA. The equation used for calculating the crystallinity $C[\%]$ of the polymer is given below:

$$C[\%] = \frac{\Delta H_m}{\Delta H_m^0} \quad (2)$$

where ΔH_m is the enthalpy of fusion of the studied sample and ΔH_m^0 is the enthalpy for 100% crystalline PLA samples, assuming that no cold crystallization takes place during the heating run. Otherwise, the cold-crystallization enthalpy should be subtracted from the melting enthalpy (Müller et al. 2015). Depending on the molecular weight and amount of L-, D-, or meso-lactide in the main chain, the polylactides can be either amorphous or semicrystalline at room temperature. PLA resins with a higher concentration of D-lactic acid show a lower tendency to crystallize (Hamad et al. 2015). Figure 4 DSC curves PLA films with different D-lactide contents. PLA film with 1.4% D-lactic acid exhibited the lowest cold crystallization temperature at 104.1 °C with a sharp and narrow exothermic peak. The films showed a lower tendency to crystallize on increasing the D-lactic acid

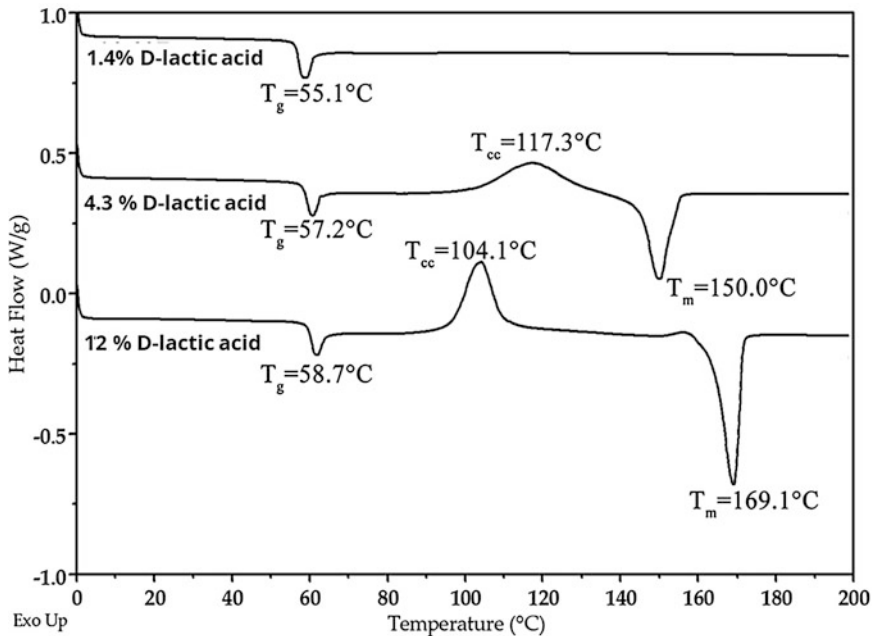


Fig. 4 DSC curves of the PLA films with different D-lactic acid contents. (Reproduced from Pölöskei et al. 2020)

content to 4.3% which is represented by a wide cold crystallization exothermic peak at 117.3 °C. PLA films with the highest D-lactic acid content (12%) did not show any tendency to crystallize (Pölöskei et al. 2020).

High molecular weight, on the other hand, can reduce the crystallization rate and thus the degree of crystallinity (Casalini et al. 2019; Jiménez et al. 2014; Müller et al. 2015). A decrease in percentage of L-lactic acid and increase in percentage of the D-isomer are observed to decrease the crystallinity of the polymer. The relationship between the T^∞ and the concentration of D-lactide (X_D) can be given by the following equation (Saeidlou et al. 2012):

$$T^\infty = \frac{13.36 + 1371.68 X_D}{0.22 + 24.3X_D + 0.42X_D^2} \quad (3)$$

Rheological Properties

The rheological properties of PLA and its blends and composites have been investigated extensively by different characterization techniques (Fang and Hanna 1999; Hamad et al. 2010, 2011, 2012; Huneault and Li 2007). Dorgan and co-workers investigated Schulz-Blaschke and Mark-Houwink constants for dilute PLA with 0–20% D-lactic acid and different chain lengths in solvents like chloroform,

tetrahydrofuran, and a mixture of acetonitrile and dichloromethane. Their findings showed that the relative percentage of the optical co-monomers in the PLA chain did not impact the fundamental rheological parameters. The viscosity of branched PLA was higher than that of linear PLA in the Newtonian range, which implies that PLA behaves as a linear polymer with random coil conformation in the tested solvents (Dorgan et al. 2000). Chile et al. studied PLA with controlled stereochemical configuration and concluded that only the isotactic chain of PLA developed higher viscosity than the other types of configurations (Chile et al. 2016). Kim et al. revealed that branched and starlike structures decreased the intrinsic viscosity of PLA compared with the linear polymer chain of equivalent molecular weight (Kim et al. 2004). The dependence of hydrodynamic radius on molecular weights in solvents like tetrahydrofuran has been investigated extensively by various authors. The values obtained were characteristic for random coil polymers in good solvent (Othman et al. 2011, 2012).

To understand the processability and flow during melt processes, study of the viscoelastic behavior as a function of temperature is useful. Due to the low thermal stability of PLA during rheological measurements, PLA often fails to comply with the time-temperature superposition principle. Palade et al. developed a method, now often employed, using tris(nonyl phenyl)phosphite (TNPP) as a stabilizer to suppress thermal hydrolysis of PLA. PLA exhibited Newtonian behavior at the low shear rates ($<10 \text{ s}^{-1}$), whereas it exhibited shear thinning at the high shear rates ($>10 \text{ s}^{-1}$) (Palade et al. 2001). Numerous studies have described the rheological behavior of PLA and indicated that PLA obeyed the power law over a certain range of shear rates and temperatures in the same way as other polymers. The tacticity of the macromolecules had some impact on the melt rheology, where isotactic PLA had a higher zero-shear viscosity and syndiotactic PLA had a lower zero shear viscosity than heterotactic PLA (Hamad et al. 2014; Saeidlou et al. 2012; Shin et al. 2010; Wang et al. 2011). The increase in melt viscosity can be achieved by stereocomplex technology. The connection between stereocomplexed domains could be varied from chain entanglement to direct molecular bridging by changing the content of stereocomplex (from 10 to 23%) (Ma et al. 2015).

Mechanical Properties

PLA has better mechanical properties, especially tensile elastic modulus and tensile and flexural strength, in comparison with conventional polymers like polystyrene or polyethylene. However, they have a low elongation at break and impact strength which limits their applications in areas requiring deformation at a higher stress (Farah et al. 2016; Hamad et al. 2015; Sanivada et al. 2020).

Mechanical properties of PLAs can be improved by plasticization, copolymerization, or blending with other biodegradable polymers (Cheng et al. 2009; Liu and Zhang 2011; Zou et al. 2021). It has been observed that the mechanical properties of PLA are to a great extent dependent on their molecular weight, stereochemistry, and crystallinity. Semicrystalline PLAs are shown to exhibit better mechanical properties

in comparison with their amorphous counterparts. Semicrystalline PLA has a tensile strength of ~50–70 MPa, a tensile modulus of 3–4 GPa, a 2–10% elongation at break, a flexural strength of 90–100 MPa, and a flexural modulus of 4–5 GPa (Farah et al. 2016; Ilyas et al. 2022; Perego and Cella 2010). Many researchers have looked into the relationship between PLA mechanical characteristics and its molecular weight. A 20% increase in tensile strength was observed by Engelberg and Kohn on increasing the Mw of the polymer from 107 to 550 Kg/mol (Engelberg and Kohn 1991). The variation in mechanical properties relative to molecular weight becomes less pronounced at higher molecular weight. PDLA and amorphous PLLA exhibited different tensile and flexural properties in a selected range of molecular weights, which can be attributed to the stereochemical makeup of the polymer backbone. Hence, by manipulating the molecular weight, stereochemistry, and crystallinity of PLA, one can tune the mechanical properties of PLAs from soft elastic materials to stiff, high-strength materials (Farah et al. 2016; Hamad et al. 2015; Perego and Cella 2010).

Biodegradation

Degradation of polymers generally occurs by scission of their main or side chains. This can be induced by hydrolysis, enzyme action, photolysis, oxidation, or thermal activation (Madhavan Nampoothiri et al. 2010; Zaaba and Jaafar 2020). Polymers containing hydrolytically unstable linkages like esters, anhydrides, or amides will undergo biodegradation (Saad and Suter 2001). PLAs contain ester linkages in their backbone and can undergo biodegradation. PLA biodegradation occurs in two stages: first hydrolysis of the ester bonds and then enzymatic breakdown of lower molecular weight fragments (lactic acid or water and carbon dioxide). The rate of hydrolytic breakdown is mostly determined by temperature, pH, and humidity (Rudnik 2013; Siakeng et al. 2019; da Silva et al. 2018; Tokiwa and Calabia 2006). Degradation of PLAs under natural conditions has not been successful. Soil burial tests of PLLA showed that they were not degraded even after three years (Calabia et al. 2010). A study by Tsuji and co-workers showed that blended films of PLLA and poly(ϵ -caprolactone) had better biodegradability than the neat polymer itself (Tsuji et al. 1998). Several investigations have shown that biodegradability of PLAs can be improved by mixing them with natural materials like cellulose and starch (Cheung et al. 2010; Kalita et al. 2021; Wan Ishak et al. 2020; Yu et al. 2020). Brdlik et al. demonstrated the improvement in biodegradability of PLA on incorporating natural-based plasticizers like acetyl tributyl citrate (ATBC), CaCO_3 , and lignin-coated cellulose nanocrystals (L-CNC) into the polymer. Figure 5 shows SEM images of the surfaces of neat PLA film and PLA films with different additives after processing and after three months of biodegradation (Brdlik et al. 2021). The surface of the neat PLA films was relatively smooth, with a small number of cavities. PLA films with plasticizer, on the other hand, had a rough surface with many eroded pinholes which is an indication of higher biodegradation rates of these films compared to a pure PLA film.

The slow degradation rate of PLA under natural conditions is due to the low population of microorganisms capable of degrading PLA in the environment

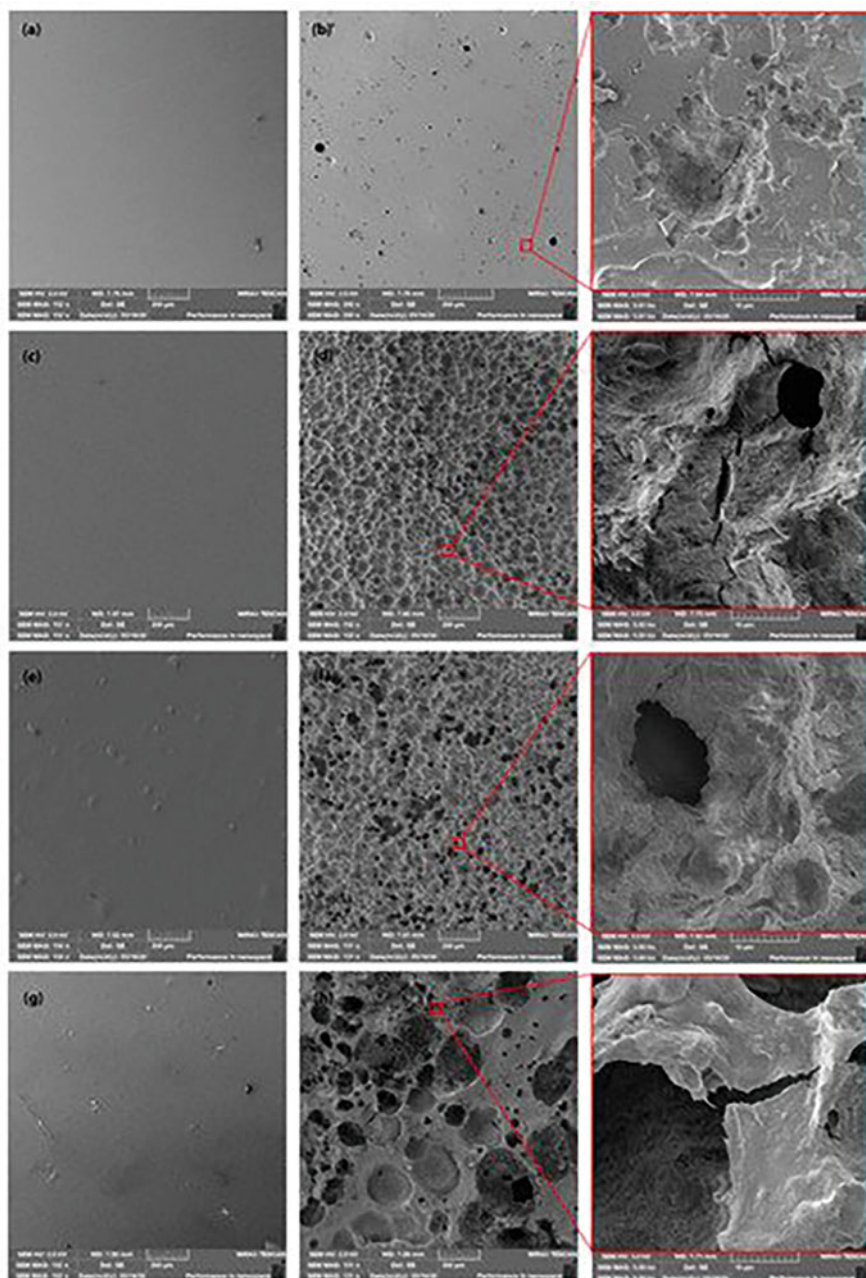


Fig. 5 SEM images of (a) neat PLA film after processing, (b) neat PLA film after biodegradation, (c) PLA/ATBC film after processing, (d) PLA/ATBC film after biodegradation, (e) PLA/ATBC/L-CNC film after processing, (f) PLA/ATBC/L-CNC film after biodegradation, (g) PLA/ATBC/CaCO₃ film after processing, and (h) PLA/ATBC/CaCO₃ film after biodegradation. (Reproduced from Brdlik et al. 2021)

(Tokiwa and Calabia 2006). Nevertheless, degradation of PLAs can be achieved by different microorganisms under controlled conditions. PLAs degrade within a period of a few weeks to months in a compost, where the temperature and humidity are high (Calabia et al. 2010; Ho et al. 1999; Satti et al. 2018). *Amycolatopsis*, *Lentzea*, *Kibdelosporangium*, and *Saccharothrix* are some of the microorganisms which are capable of degrading PLAs (Jarerat et al. 2002; Sukkhum 2011). Several enzymes, including pronase, esterases, and bromelain, have been used to study their effect on PLLA degradation rate. Among these, proteinase K which is isolated from *T. album* Limber has been shown to significantly accelerate the degradation rate of PLLA (Lee et al. 2014; Sukkhum 2011; Tokiwa et al. 2009; Williams 1981). Degradation studies of PLA are of particular interest due to their applicability in the medical field to make drug delivery vehicles, implants, or surgical sutures (Baranwal et al. 2022; Calabia et al. 2010; Saad and Suter 2001).

Applications of PLA

PLA is called the “polymer of the twenty-first century” owing to its multifunctional properties. As discussed in the previous sections of this chapter, PLA is a bio-based polymer derived from renewable resources like corn, sugar cane, rice, wheat, and other starch-rich products. The FDA has approved PLA for direct contact with biological fluids. PLA on degradation gives lactic acid which is naturally present in our body and hence is compatible. Biocompatibility and bioresorbability are two qualities of PLAs which have made them popular in the healthcare sector to make active packaging materials, tissue engineering scaffolds, drug delivery vehicles, medical implants, and medical instruments. PLA is also in demand in other industrial sectors like automotive, textile, and agriculture due to its relatively low production cost and commercial availability compared to other biopolymers. PLAs can be used to prepare breathable, lightweight, recyclable fabrics. Moreover, PLA fibers have good UV resistance, inherent biological resistance, and good anti-flame property. It is the only bio-based biodegradable polymer which can be melt-spun into strong textile fibers on a large scale (Yang et al. 2020). They also have gained interest in the packaging industry to make compostable eco-friendly packaging materials. PLA exists in a glassy state at room temperature which prevents the movement of large molecules through the polymer matrix. Hence, PLA materials are excellent barriers to aroma, flavor, or other large molecules. Thus, PLA packaging can protect the product inside from extrinsic elements like moisture, unwanted odors, and other contaminants. It also prevents the odor or flavor molecules of the product to escape the package, thereby keeping the product fresh (Whiteman et al. 2001). PLA-based products have several advantages such as good thermomechanical properties, durability, corrosion resistance, lightweight, ease of processability using existing techniques (injection molding, extrusion), and relatively low cost. As a result, PLA plastics are becoming more appealing for long-lasting applications such as electrical, electronic, and automotive products, as well as mechanical components that require high-performance materials with low environmental impact.

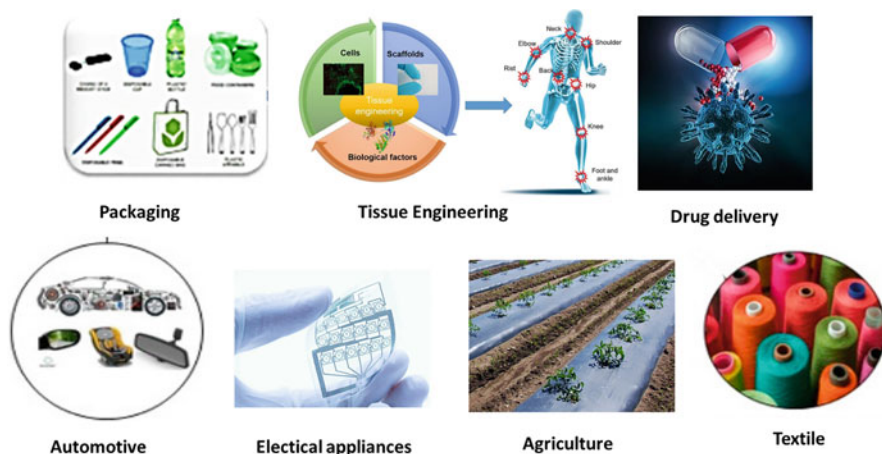


Fig. 6 Various applications of PLA

In many research works, PLA is blended with other biopolymers or composites to attain the desired features. It can be formulated to be both rigid and flexible to suit different applications. Both physical and chemical features of PLAs may be further enhanced by surface modification. The following sections cover the applications in tissue engineering, drug delivery, and so on. Figure 6 gives the various applications of PLA.

Biomedical Industry

Biomedical industry demands biocompatible materials for numerous applications. Nowadays, traditional biocompatible materials are substituted by biopolymers in many fields. In addition to biocompatibility, biodegradability of these biopolymers is also a significant feature that attracts researchers to use them in biomedical devices. PLA is an important polymer possessing these properties. Degradation of these biopolymers results in nontoxic products that can be easily eliminated from the body by means of usual cellular functions (Uzun et al. 2015). Bioabsorbability of PLA is also highly advantageous when used in fixation devices as dissolvable suture meshes. It can overcome many of the limitations of metallic implants. During bone implants and scaffoldings, a second surgery can be avoided by the usage of bioabsorbable polymer such as PLA as the implant material. A gradual recovery by the regeneration of the tissues is possible along with the degradation of the device.

Tissue Engineering

Tissue engineering is an interdisciplinary field that finds a solution to organ failure and tissue loss. It aims at restoring injured tissues, thereby improving their functions. Earlier, metals have been used as implants and in regenerative medicine. But the usage

of metals is limited due to their lack of biodegradability. These metals are nowadays replaced with biopolymers, which offer better biodegradability and biocompatibility.

Cell proliferation and growth demand temporary support known as a scaffold or template. When cell growth and regeneration happen, the scaffold slowly degrades along with the development of the new tissue. Current research suggests that biomaterials are an important choice to act as scaffolds. Easy processing, cell adhesion, porosity, biocompatibility, and biodegradability are the major characteristics for a biomaterial to act as an excellent scaffold. Such scaffolds should never induce any inflammatory or cytotoxic responses in the body (Monnier et al. 2018). Also, degradation products should be easily metabolized and removed from the body. The mechanical properties of the scaffold should be similar to those of the restored tissue. Biomimetic structures can be successfully fabricated by 3D printing. It is a promising technology through which bone regeneration can be achieved. Synthetic polymers have been widely chosen for 3D printing owing to their excellent mechanical properties and easiness in fabrication. In the case of the synthetic polymer PLA, ductility and toughness may be enhanced by copolymerization and the formation of nanocomposites. Many research works have been proposed to generate scaffolds for bone tissue engineering by the copolymerization of PLA with hydroxy apatite, polyethylene glycol, and so on. Such copolymerization enhances the mechanical properties, which is highly beneficial in the case of tissue regeneration. Along with good mechanical properties, bioactivity is also essential in generating new tissues. It can be achieved by surface modification of the polymer by proteins or peptides, DNA molecules, and so on. As a result, cell adhesion and proliferation can also be enhanced. In a recent work, bone-regenerating 3D scaffolds were prepared from PLA and gelatin with mucic acid. It was found that the physicochemical properties were improved compared to the neat synthetic polymer. The viability of mouse mesenchymal stem cells remained unaffected when seeded onto this PLA/gelatin/mucic acid scaffold. This proved that the 3D printed PLA scaffolds coated with gelatin and mucic acid have potential applications in bone tissue engineering (Velioglu et al. 2019).

3D printing is a brilliant methodology to fabricate macro- and microporous structures from polymers, metals, and ceramics. Among 3D printing techniques, fused deposition modeling is a prominent one for fabricating scaffolds. Bone tissue engineering is a complicated process since many factors such as pore size, shape, mechanical properties, biocompatibility, and biodegradability are taken into consideration during regeneration. Fused deposition modeling is utilized to develop PLA scaffolds containing polydopamine and collagen I. In the synthetic method, PLA scaffolds are dipped in dopamine solution followed by the conjugation of polydopamine. When polydopamine is coated, it assisted in the coupling of collagen onto the PLA scaffolds. High cell density on the scaffold controlled the level of F-actin cytoskeleton and vinculin adhesive plaques (Teixeira et al. 2019). A similar work was done by Ritz et al. by developing PLA scaffolds loaded with collagen I and stromal-derived factor-1 by means of fused deposition modeling. It produced both disc-like and cage-like scaffolds, and they could promote endothelial cell growth. The results prove that these scaffolds have potential applications in bone tissue

engineering (Ritz et al. 2017). The same synthetic method was used by Oladapo et al. to prepare the PLA/hydroxyapatite composite. These scaffolds possess a cylindrical shape that mimics the architecture and structure of the bone. Also, the ability of bone regeneration and bioactivity can be enhanced by the incorporation of hydroxyapatite in PLA (Oladapo et al. 2019). In another work, phenylenediamine functionalized carbon dots were composited with silk fibroin and PLA to obtain bioactive scaffolds. A uniform distribution of carbon dots was obtained, which improved mechanical, physical, and chemical properties. These nanofibrous porous scaffolds displayed an improvement in young modulus and good efficiency in implantation. These prepared scaffolds were implanted into rat cardiomyocytes, and the cell viability was significantly improved when made into a composite with carbon dots. This proves its potential to act as well organized scaffolds for cardiac tissue engineering (Yan et al. 2020).

Scaffolds prepared from nanofibers of PLA have been widely used in regenerative medicine. The high surface area, ability to mimic living cell architecture, and tunable mechanical properties of these scaffolds offer a design apt for tissue regeneration in the body. PLA is often opted for tissue regeneration since it has good thermal processability (Farah et al. 2016). But the brittleness of PLA is an important drawback to be considered. Modification of PLA may be performed to improve chemical inertness, hydrophilicity, degradation rate, and toughness. PLA nanofibers act as potential scaffolds for nervous, cutaneous, cardiovascular, and musculoskeletal tissue engineering. The most significant factor in the construction of scaffolds is cell adhesion. To improve mechanical properties and cell adhesion, scaffolds have been fabricated by different methods such as melt mixing, leaching, electrospinning, and so on. Blending with different polymers and composite formation also modify the properties. For bone tissue engineering, 3D printing is considered as a recent procedure to develop a porous scaffold (Roseti et al. 2017). Such scaffolds could provide strong osteoinduction activity and fast vascularization. In bone tissue engineering, it is very essential that the engineered bone must integrate completely with the native bone and surrounding environment. The lack of such integration may lead to many complications in physiological functioning. Implantation of such a biomaterial may not be able to achieve the expected result of tissue regeneration and immune response. An excellent result was observed when PLA was blended with hydroxyapatite. In this case, each component in the blend affects the features of the other components (Bae et al. 2011).

Mechanical properties, porosity, biodegradability, and bioactivity influence the quality of a scaffold. PLA/calcium phosphate composites have been prepared to integrate into host tissues, and it was seen that these porous scaffolds can be successfully utilized for the proliferation of osteoblasts. This modified scaffold could bring about a better cell adhesion as the pure PLA offers a poor adhesion to the cells. In addition, porosity also improved to 97% from 93%. These composites established superior mechanical properties when compared with the pure PLA scaffold. Hassanajili et al. proposed an indirect 3D printing to fabricate bone scaffolds using PLA/PCL/HA composite. The porosity of the scaffold was found to be 77%, and the mechanical properties were comparable with the normal tissue. The

in vitro studies on the osteoblast cells found that the excellent cell adhesion, as well as cell proliferation of the scaffold, makes it a potential candidate for bone tissue engineering (Hassanajili et al. 2019). Figure 7 shows the demonstration of PLA/PCL/HA scaffold for osteoblast cells.

In a recent work, the electrospinning technique was used to prepare zeolite-hydroxyapatite blended PLA/PCL nanofibers for dental tissue engineering. Ring-opening method was used for PLA/PCL polymerization, and zeolite and hydroxyapatite were prepared via hydrothermal synthesis. Cell viability was tested by the MTT assay, and it was seen that blended nanofibers act as potential scaffolds for dental tissue regeneration (Mohandesnezhad et al. 2020). Figure 8 demonstrates the electrospinning method for the preparation of nanofibers from zeolite-nHA blended PCL/PLA for dental tissue engineering.

Table 1 shows various PLA scaffolds for tissue engineering. The aim of tissue engineering is to develop scaffolds for the regeneration of damaged tissues. Recent studies prove that nanofibers are well suited to form porous structures that can provide mechanical support as well as mimic the normal tissue. PLA is a synthetic polyester, and its degradation rate can be altered by changing the structure, porosity, molecular weight, viscosity, and crystallinity. PLA and its blends and composites have been widely studied for various types of tissue engineering.

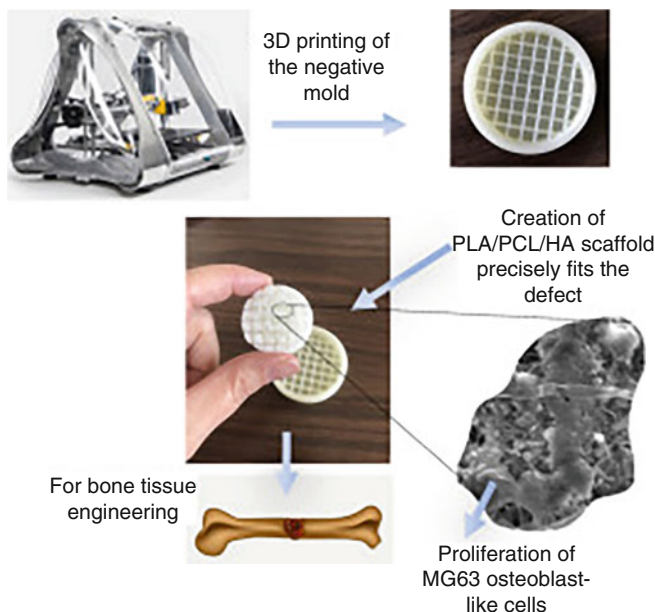


Fig. 7 PLA/PCL/HA scaffold for osteoblast cells. (Reprinted with permission from Hassanajili et al. 2019)

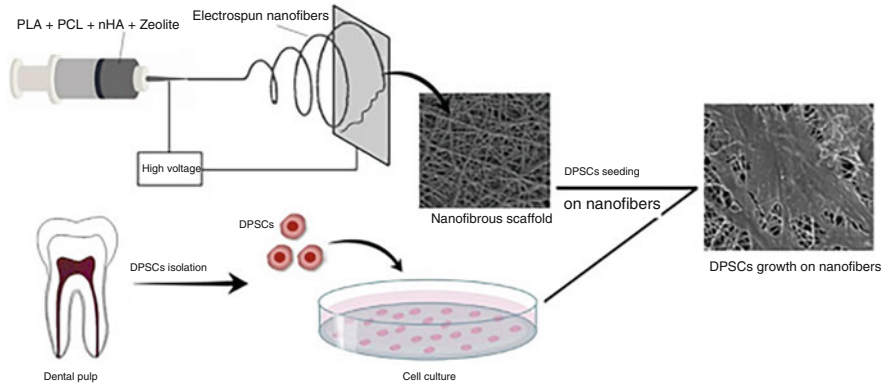


Fig. 8 Zeolite-nHA blended PCL/PLA nanofibers for dental tissue engineering. (Reprinted with permission from Mohandesnezhad et al. [2020](#))

Table 1 Different PLA scaffolds for tissue engineering

Sl. no.	PLA scaffold	Type	Application	References
1	PLA/PCL	Dental	Proliferation of human dental stem cells	Mohandesnezhad et al. (2020)
2	PLA/HA nanocomposite	Musculoskeletal	Better ceramic-polymer interactions	Z. Fang and Feng (2014)
3	PLA/silk fibroin	Nervous	Neuronal cell differentiation, neurite extension	Tian et al. (2015)
4	PLA/gelatin	Cardiovascular	In vitro culture with endothelial cells and muscle cells	Shalumon et al. (2015)
5	PLA (mineralized)/strontium	Bone	Promoting bone defect repair	Han et al. (2019)
6	Electrospun PLA	Cutaneous	In vitro test on human dermal keratinocytes and fibroblasts; in vivo implant mouse skin model	Mohiti-Asli et al. (2017)
7	PLA scaffold	Bone	Osteosarcoma cell proliferation	Gregor et al. (2017)
8	PLA scaffold	Dermal	Biocompatibility on human dermal fibroblasts	Karabay et al. (2019)
9	PLA/hydroxyapatite	Bone	Proliferation and differentiation of osteoblastic cells	Carfi Pavia et al. (2018)
10	PLA/hydroxyapatite/lignocellulose/bioactive glass	Bone	In vitro biomineralization studies	Mao et al. (2018)

Drug Delivery

The efficiency of a drug is mainly assessed by the responses given by the body after its administration. Bioactive carriers are usually chosen as drug delivery system, and PLA is a good choice owing to its features. Degradation rate is also a determining factor in the case of drug delivery systems. The desired effect can be obtained by altering the degradation rate. For a constant, timely, and continuous release of the drug, the rate of breakdown of the drug carrier plays a prominent role. In some cases, this drug release should be prolonged for a sustainable drug release. Such a drug release assists in providing sufficient time so that the expected result may be obtained. Also, in some cases, targeted drug delivery at some specific sites is essential for the better action.

Polymeric nanofibers are widely used for many biomedical purposes among which the encapsulation of antitumor drugs for drug delivery applications covers a major area. Polymers possessing stimuli-responsive abilities and biocompatibility are well suited for this purpose. PLA is an excellent material because of its good biodegradability and dissolution in the extracellular environment. The FDA (US Food and Drug Administration) has approved the usage of PLA as a biomaterial (Liu et al. 2020b). PLA scaffolds possess nanofibers of variable pore diameter, which permits the delivery of different types of drugs. A controlled drug release is also possible by this characteristic nature (Martin et al. 2019).

Electrospinning is a widely accepted technique to obtain nanofibers from polymeric materials. Electrospinning always produces fine fibers with a large surface area, which assists in improving the properties. It can be used to produce nanofibers with a high surface-area-to-volume ratio which supports efficient drug delivery. Before loading the drug into the polymeric material, dissolution should be tested. Drug can be dissolved directly, if both the polymer and drug are soluble in the same solvent. Otherwise, another solvent is used for solubilizing the drug. Drug-releasing mechanism proceeds via desorption of drug from the surface of nanofiber. Nanofiber diameter, morphology, and porosity are the different parameters regulating the drug-release kinetics (Fig. 9).

The design of nanofiber-assisted drug release systems depends on both the nature of the drug and the purpose of drug delivery. Such a delivery mechanism can be either by immobilization on the nanofiber surface or through encapsulation. Figure 10 depicts these two mechanisms of drug delivery. The quantity of drug release depends on various factors such as the interaction between the drug and the carrier, nanofiber diameter, and the drug content (Cheng et al. 2018). In a recent study, polylactic acid-hydroxyapatite-doxycycline nanofibers obtained via electrospinning have been used as the drug carrier. Drug-releasing abilities were tested in phosphate buffer solution and simulated body fluid. In vitro drug release analysis and its kinetics confirmed that 3 and 7% of the drug-loaded samples obtained via physical adsorption are acceptable systems for prolonged release of drugs (Farkas et al. 2022).

It is significant to note the physicochemical processes that affect the drug release rate and steps in the release mechanism before developing a drug carrier. The various ways of drug release may happen while passage through the polymer, water-filled pores, or by dissolving in the encapsulated polymer. In the case of PLA, water-filled pores are the most convenient way. Various mechanisms

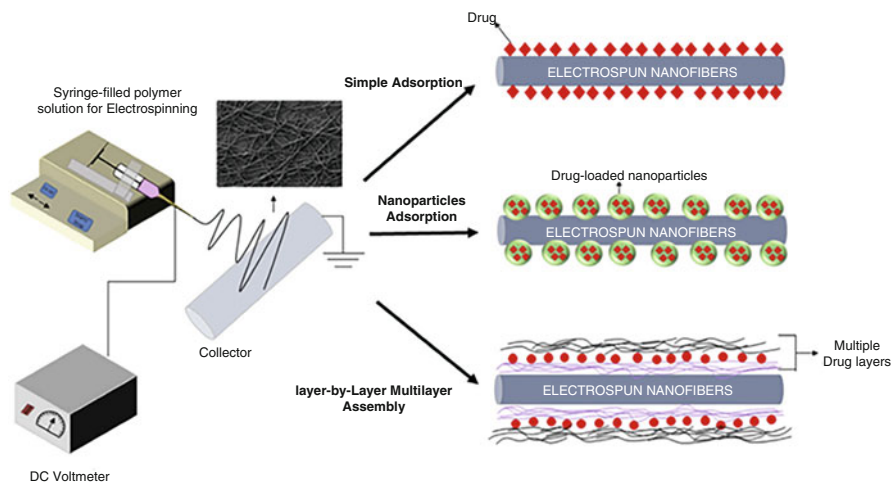


Fig. 9 Electrospun nanofibers for drug delivery. (Reprinted with permission from Stack et al. 2018)

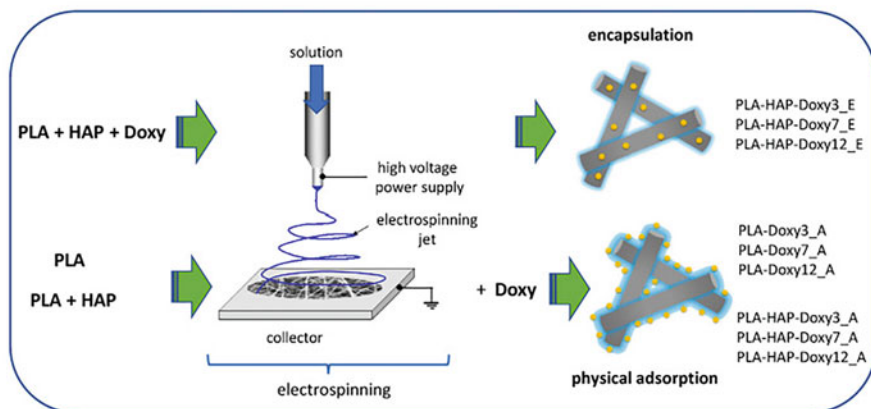


Fig. 10 Two different routes of drug loading. (Reprinted with permission from Farkas et al. 2022)

involved are diffusion from the surface and diffusion of particles at the time of degradation of the polymer, through swelling of matrix. These mechanisms are depicted in Fig. 11.

Packaging

Plastics have been considered as the chief source of packaging material for decades, and synthetic plastics are heavily consumed by the packaging industry. But these packaging materials leave a bulk amount of waste which pollutes the environment,

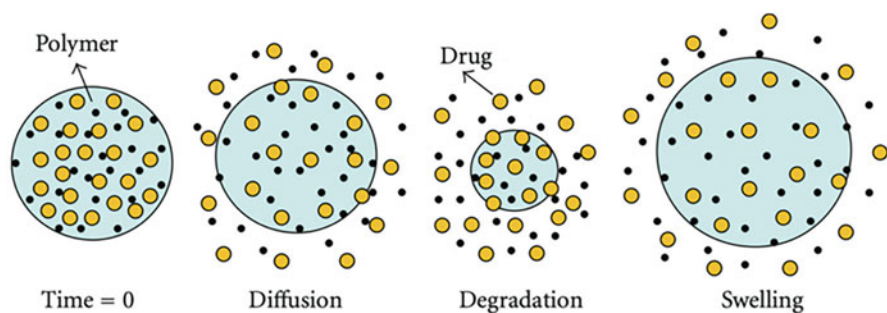


Fig. 11 Mechanisms of drug release. (Reprinted with permission from Vlachopoulos et al. 2022)

and now it's a real threat to the existence of life. So, it is a necessity to replace conventional plastics with biodegradable materials that can function as packing materials. Biopolymers are a good choice of green materials which can serve the purpose. Among these biopolymers, PLA is a well attracted one, but some disadvantages limit its performance. Fast hydrolytic degradation is one such drawbacks which limits its application as a food packaging material. It is important to improve the barrier properties against moisture while acting as a packing material. To enhance the barrier properties, several methods may be adopted. Coating of biopolymer with materials which improve hydrophobicity is a method to overcome the issue. But these drawbacks can be successfully nullified by blending PLA with natural fillers making composites. PLA-polyethylene glycol and PLA-polycaprolactone blends are some examples.

While used as food packaging materials, in addition to biodegradability, both flexibility and hardness are significant parameters for excellent handling. In the form of film or food tray, the packaging material should ensure sealability. PLA and its blends are well known to be used as food tray and packaging films as they possess the abovementioned features. Technological parameters should be optimized to regulate the processing features and are varied according to their applicability. Figure 12 shows the various steps for obtaining food packaging system from biopolymers.

Automotive Applications of PLA

Plastic materials cover a major portion of automotive applications, and currently, composites of synthetic polymers are in high demand in this sector on account of the reduction of vehicle weight upon their usage. Composites containing organic fillers are found to be easy to recycle. Hence, environmental concerns also favor the utilization of such bioplastics and their composites. Recently, natural fibers are composited with polymers like PLA to attain a biodegradable matrix for use as automobile parts. In such cases, measures have to be taken to enhance the interfacial adhesion between fiber and polymer matrix to achieve the best results. Ductility and



Fig. 12 Steps for obtaining food packaging system from biopolymers and blends (Darie-Niță et al. 2021)

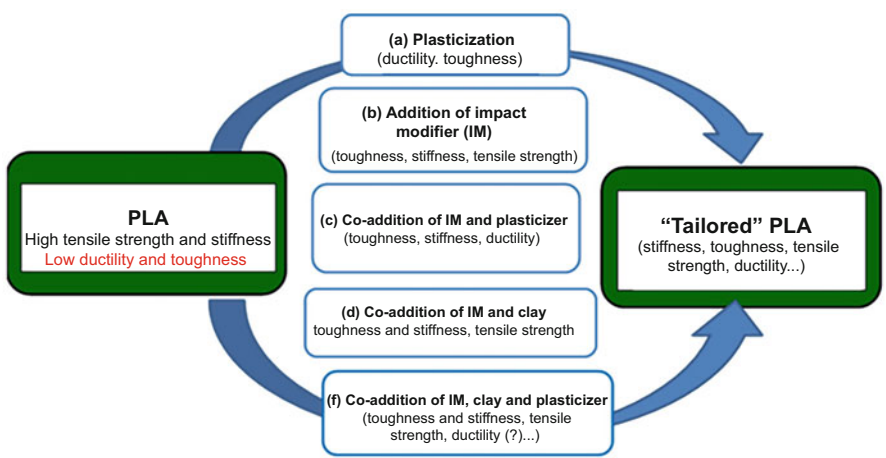


Fig. 13 Tuning PLA features for automotive applications

toughness also need to be improved, which is generally attained by plasticization (Balieu et al. 2013). Among the various plasticizers, those with good mobility and high molecular weight are usually preferred. As seen in Fig. 13, different methods may be adopted to enrich the properties of neat PLA.

Textile

PLA being biodegradable, it finds many applications in textile industry. The degradation process is also comparatively easy as it needs only cleavage of ester linkage.

Even though it is biodegradable, mechanical properties are comparable to plastics. It can be molded into decided shapes with a variety of features, as in the case of other plastics. Other parameters such as renewable origin, recycling possibilities, and ease of melting also make PLA an acceptable material in the textile industry. Thermal stability and resistance to shrinkage during ironing were also found to be highly advantageous while PLA stereocomplexes were formed. Another property is its comfort, as in the case of other natural fibers like silk, cotton, and so on. These properties make PLA fibers excel in various textile applications.

Spinning can be done to process PLA into fibers. Organic compounds can be absorbed easily, and this property can be utilized in fiber applications. It also has the ability to absorb moisture due to its polar nature, which makes it a good candidate for wiping applications. Potential wicking features of PLA can be used to generate disposable products. These properties of PLA increase its use in the textile industry, and nowadays it replaces many of the synthetic polymers such as polyethylene terephthalate and nylon. The use of PLA-cotton blends is wide in garment industries, and it is found that its buffering ability to sweat and thermal insulating properties enhance its usage in sports. Good resiliency of PLA is utilized in the manufacture of jackets also. Good crimp and retention features of PLA make it suitable for embroidered fabrics. PLA also finds applications in homeware products such as pillowcases and curtains. These significant features of PLA prove its potential in textile and fiber applications. Dyeing processes are done by varying different parameters such as pH, temperature, and time. It is a challenge to attain the suitable temperature in finishing as PLA is easily susceptible to degradation. Another limitation of PLA textiles is that their ironing temperature is comparatively lower than conventional textiles like cotton.

Some PLA composites were fabricated to enhance productivity and reduce waste to obtain some tailored products in textile industry. For instance, carbon black and carbon nanotube are composited with PLA to improve the adhesion properties for fabricating functional fabrics. In many works, 3D printing is done to develop such products. In such synthetic procedures, the various parameters of 3D printing, such as printing speed and temperature, influence the adhesion strength. Comfort and durability of a textile manufactured from polymeric composite materials are the major challenges during 3D printing. In a work, 3D printing was adopted to obtain PLA-carbon deposited into polyethylene terephthalate. The results show that tensile properties were affected during composite formation due to diffusion of the polymer, and that in turn led to a weak adhesion.

Agriculture

The use of flexible plastic films in the agriculture sector has increased immensely in the past few decades. Plastics like polypropylene and ethylene-vinyl acetate copolymer have been widely employed as greenhouse films, mulch films, silage films, stretch films, etc. This has helped the farmers in improving their crop quality, obtaining higher yields, minimizing water consumption, and reducing their ecological footprint (Rocha et al. 2018). According to a report by Sintim and Flury, plastic

mulch films accounted for approximately 40% of the total plastic films used in agriculture (Sintim and Flury 2017). Plastic mulch helps to conserve soil moisture, reduces the loss of minerals, improves fertility of the soil, minimizes weed growth, protects the plants from insect attack, and keeps the fruits and vegetables clean. However, the incorrect post-use disposal of these nonbiodegradable plastic films poses a serious threat to the environment. Biodegradable mulch films are a superior alternative to plastic mulch films and can be discarded into the soil to be degraded by the action of microorganisms. This eliminates the cost and labor associated with the removal and disposal of plastic mulch and is safer for the environment. PLA, which can be composted without leaving any harmful residues in the soil, is a good option to prepare mulch films. PLA is blended with other polymers or fillers to improve their flexibility, and these blends are more suited for agricultural applications than virgin PLA. PLA-based plastic films can also be used to manufacture biodegradable sandbags, weed prevention nets, vegetation nets and pots, etc. (Malinconico et al. 2018; Rocha et al. 2018).

Jandas et al. reported the preparation of completely biodegradable agricultural mulch film made from PLA/poly(hydroxybutyrate) blend with maleic anhydride as the reactive compatibilizer. Flexibility and impact modification were considerably enhanced by the addition of poly(hydroxybutyrate) and maleic anhydride. The films were reinforced with nano-clays to improve their tensile strength and tensile modulus without affecting their ductility. These films also showed an increased rate of biodegradation compared to virgin PLA. Environmental sustainability studies as well as mechanical testing showed that these films could be used for short-term crops that can be completed within around 100–150 days (Jandas et al. 2013b).

Calcagnile and co-workers developed a completely biodegradable composite material from PLA and cellulose-based super-absorbing hydrogel. This material is capable of acting as a source of water and fertilizers and can release them into the soil in a controlled manner. This allows for more efficient management of water and fertilizer and causes less chemical damage to the plants (Calcagnile et al. 2019). Durpekova et al. also reported a similar super-absorbing hydrogel prepared by blending low molecular weight PLA, acid whey, and cellulose derivatives, which could be employed as a reservoir of water and nutritive whey agents for crops and also for measured release of fertilizers (Durpekova et al. 2022).

Paschoalin et al. developed a wearable electrochemical sensor based on PLA for the detection of bipyridinium and carbamate pesticides on the surface of food and agricultural samples. This economic device consists of a three-electrode system which is deposited on solution-blow spinning mats of PLA by screen-printing technology. The flexibility of these sensors allows them to be on flat, curved, and irregular surfaces of leaves, fruits, and vegetables. The researchers could attain a detection limit of 57 nM and 43 nM for diquat and carbendazim, respectively, using this device. This sensor could also differentiate and measure the amount of carbendazim and diquat on cabbage and apple skins without any interference from other pesticides (Paschoalin et al. 2022).

Crop mulching promotes root development by promoting soil warming. This process reduces the usage of herbicides and improves production quality. In

traditional agricultural methodologies, low-density polyethylene is used as mulching materials. The good mechanical and optical properties of these conventional materials enhance the growth of agricultural crops. But, when environmental sustainability is concerned, the exceptional mechanical properties and long life of these mulching materials pose a threat. Various climate agents spread these materials to different locations, and these residues may in turn cause contamination of the soil. Poor agricultural methods and repeated use of these mulching agents cause adverse effect on the environment. Hence, it was a necessity to replace these conventional mulching agents with some biodegradable materials in a reliable method. Degradation of a mulching agent which should be done in a fast time is the solution to the problem. In such a case, it is possible to rely on biodegradable PLA and its derivatives to avoid such issues. Complete degradation of PLA films is possible by the activity of soil bacteria if there is moisture content in the soil.

Electronic Appliances

The upgradation of electronic devices at a very fast pace is resulting in a huge accumulation of electronic waste. The use of plastic materials for preparing lightweight flexible electronics is adding up to this crisis. Developing greener electronic devices using degradable polymers is a solution to this problem. Among bio-based polymers, PLAs are potential candidates for the manufacture of electronic appliances (Shi et al. 2017). However, reports on the use of PLA for making electronic devices are limited in the literature. This may be due to their low glass transition temperature, brittleness, low impact strength, low heat resistance, and unsatisfactory dielectric properties. Properties of PLA can be altered through orientation and annealing. This increases the crystallinity, mechanical performance (increased strength, stiffness, and ductility under tension), and thermal stability of PLA films. Addition of nucleating agents such as microcellulose, talc, and nanocomposites can also enhance the mechanical strength and crystallinity of PLA (Luoma et al. 2021). The heat resistance of PLA can be increased by blending with resins having a higher thermal resistance like acrylonitrile butadiene styrene (ABS) and polycarbonate (PC). Integration of glass fiber, plant fiber, and inorganic filler particles into the PLA matrix or the use of stereocomplex of PDLA and PLLA can also improve the thermal resistance of PLA. The effects of metal hydroxides and phosphorus reagents on the flame-retardant property of PLA have also been investigated (Obuchi and Ogawa 2010).

Some major companies have adopted PLA as parts of their electronic gadgets. For example, Sony used the injection-molding technique to make the casing of its Walkman in 2001. Sanyo Marwick Media manufactured CD/DVD discs and cases made of PLA. Toshiba and Samsung used PLA for their remote control and phone chassis. Fuji Xerox successfully used PLA-based interior drum cover for several copying machines in 2007. PLA was used by Fujitsu and PEGA for the hard shell case of their notebooks (Obuchi and Ogawa 2010).

Recently, Luoma et al. studied the performance of the different grades of PLA films in printed electronics processing. They observed that orientation and annealing

increased the crystallinity of the PLA films and enhanced their mechanical and thermal properties. These PLA films were used as eco-sustainable substrates for functional light-emitting diode foils with screen-printed silver conductors and die-bonded LED chips (Luoma et al. 2021).

Utilizing the flexibility, biocompatibility, and biodegradability of their own PLA, Prontera et al. showed that it is possible to produce optoelectronic devices on PLA layers. The PLA substrates prepared by melt extrusion showed ~90% optical transmittance in the visible region and 12 nm surface roughness, which are suitable parameters for OLED applications. Using hybrid technology, various structures were created on top of the PLA substrates using solution-based and thermal evaporation deposition techniques. Biocompatibility assays of the devices prepared showed that PLA is a strong contender for making bioelectronic devices (Prontera et al. 2022).

Carbon nanotube-loaded poly(lactic acid)/ethylene-vinyl acetate copolymer blends were used to make broadband microwave absorbing materials by Soares and co-workers. These composites showed excellent microwave absorption properties and a wide absorption bandwidth at low concentrations of carbon nanotubes and can be considered as potential candidates for flexible materials suitable for shielding electronic devices in wide frequency ranges (Lopes Pereira et al. 2022).

Sensing

Piezoelectric materials can generate electric charges on the surface when subjected to pressure or strain, converting mechanical energy into electrical energy making them attractive for sensing applications. Polymers with piezoelectric properties have the advantage of being lightweight, transparent, and flexible and can be used for the preparation of thin films. Semicrystalline PLLA and PDLA exhibit piezoelectricity when the polymer chains are highly oriented by the drawing process. Among these, PLLA is the more popularly researched stereoisomer. Racemic PDLA, which is amorphous, does not show piezoelectric effect. PLA has an inherent chiral molecular conformation which has different optical properties for each substituent group. The coordinated motion of the permanent dipoles present on the helical chain molecules of PLLA is the source and main reason for its piezoelectricity. When a shear stress is applied to the chain molecules in PLLA with a 10/3 helical structure through its methyl groups, it causes the displacement of all the atoms present in the chain. In particular, C=O bonds with large dipole moment will rotate, which changes the polarization of the entire long-chain molecule, generating the shear piezoelectricity of PLLA. PLLA and PDLA have helical structures due to the presence of chiral carbon. PLLA has left-handed helical structure, and PDLA has right-handed helical structure. As the helical structures of PDLA and PLLA have oppositely oriented clockwise and counterclockwise spirals, respectively, their piezoelectric constants have the opposite sign. Unlike poly(vinylidene fluoride), another popular piezoelectric polymer, PLLA does not exhibit pyroelectricity (ability to generate a temporary voltage on heating or cooling) (Tajitsu 2017).

Piezoelectric elements are key components of human-machine interfaces (HMIs) in new smart devices. A requirement for these materials is that they should not exhibit pyroelectricity. It is impossible to tell whether the signal comes from the pressure or the heat of the operator's hand if a piezoelectric sensor material displays pyroelectricity since it can instantly detect heat from a finger when it is used as an HMI. PLLA does not have intrinsic polarization, and hence it does not show pyroelectricity, and therefore it finds a place in developing HMIs. PLLA films also have a larger shear piezoelectric constant (~ 10 pC/N) compared to other polymers. Hence, they can sense bending and twisting motions with satisfactory sensitivity. If this unique function is utilized, PLLA can be used in sensor applications to allow intuitive control in HMI. Taking advantage of this special function, Murata Manufacturing and Mitsui Chemicals Inc. developed a TV remote control that combined a PLLA sensor with radio and power supply circuits, which could be controlled by the user's motion. The dye-sensitizing-type photoelectric cell fitted in this remote control produced enough electric power due to the high transparency of PLLA. The extra electric power generated by the photoelectric cell could be stored in a capacitor eliminating the need for batteries (Ando et al. 2012).

Murata Manufacturing Co., Ltd., also utilized piezoelectric PLLA films for designing piezoelectric PLLA fabrics having a strong antibacterial effect. This fabric is made of left-handed PLLA helical yarn (S-yarn) and right-handed PLLA helical yarn (Z-yarn). Application of stress on the fabric generates a strong electric field between the yarns which is caused by the reversal of the electric polarity of the yarns. The strong electric field resulting from the extension and contraction of the fabric is the reason for its antibacterial activity (Ando et al. 2017).

Wang et al. developed a visualized tactile sensing electronic skin (VTSES) made of poly(vinylidene fluoride-trifluoroethylene)/thin-film transistor/PLLA arrays. This device can detect static contact, dynamic force, and thermal transduction. The piezoelectric PLLA component of this device enables dynamic slide detection (Wang et al. 2022).

Dahiya and co-workers reported a wireless pressure sensing bandage based on inductor-capacitor resonant tank. This resonant tank is screen printed on piezoelectric PLLA nanofiber substrate which is connected in parallel with a planar inductor forming a LC circuit. The PLLA component provides a better conformal contact with the skin and increases sensitivity under the pressure due to its piezoelectric property. These smart bandages can be used for fast wound healing which is facilitated by the electroceutical arrangement due to piezoelectric PLLA substrate (Nikbakhtnasrabadi et al. 2022).

Conclusion

This chapter summarizes the properties and various applications in different fields. It is a promising candidate in many biomedical or industrial applications. Biodegradable, biocompatible, and bioabsorbable polymers have always attracted the attention of current researchers. Neat PLA and its blends and composites with its low cost and

excellent biocompatibility find applications in biomedical as well as packaging industry. It was observed that biomedical industry is the major area of research utilizing the features of PLA. Among the biomedical applications, tissue engineering is considered as the prominent one since PLA composites can be conveniently used to produce specific biodegradable body parts. It is expected that PLA composites will substitute conventional scaffolds in the near future. PLA and its composites owing to their exceptional properties are a promising research field in the future.

The chapter discusses the applications of PLA in numerous fields such as biomedical, automotive, textile, packaging, and sensing. Furthermore, enormous research is going on to design value-added products from PLA nanostructure. These efforts will establish the material in a wide range of applications which may throw back the sector of plastics in the near future.

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Biopolymer Films and Composite Coatings 43

Applications

Mariana Altenhofen da Silva and Andrea Cristiane Krause Bierhalz

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M. A. da Silva (✉)

Center of Agricultural Sciences, Federal University of São Carlos, Araras, SP, Brazil

e-mail: mariana.alt@ufscar.br

A. C. K. Bierhalz

Department of Textile Engineering, Federal University of Santa Catarina-Campus Blumenau, Blumenau, SC, Brazil

e-mail: andrea.krause@ufsc.br

Abstract

Facing the problem of excessive use of conventional petroleum-based plastic materials in a wide range of daily life applications is undoubtedly an urgent global issue. Researchers and organizations have driven much effort to develop novel materials such as coatings and films based on biopolymers which have emerged as a viable way to replace, at least partially, synthetic products. Biopolymers, like polysaccharides and proteins, can be obtained from marine or agro-industrial resources and also by food processing waste and by-products, which contributes to sustainability and a circular economy. Biopolymer films are pre-formed structures produced by solvent casting and extrusion procedures, while coatings are formed directly on the product surface by spraying, dipping, or spreading techniques. To enhance films and coatings properties, strategies including chemical modification, cross-linking, plasticizers addition, use of fibers and nano reinforcing materials, and blending of different biopolymers have been adopted. Although there is still much to be done in terms of mechanical and water barriers properties and large-scale processability, the incorporation of active agents into the biopolymer matrix, mainly natural antimicrobials and antioxidants, vitamins, nutrients, and growth factors, has emerged as one of the significant advantages in the design of novel functional materials. These active ingredients can prolong shelf life and provide many other additional characteristics of the final products. This chapter intends to cover the relevance and recent advances in biopolymer films and composite coatings, highlighting their applications in different sectors such as food packaging, medicine, textile, and agriculture.

Keywords

Biopolymers · Food packaging · Textile · Biomedical films · Mulching

Introduction

Owing to the scarce petroleum resources and the urgent need for more sustainable, eco-friendly, and cost-effective alternatives, biopolymers, due to their renewability and biodegradability, have emerged as promising raw materials for a wide range of materials and applications. Although a total replacement of conventional petroleum-based by bio-based plastics seems impossible, for some specific applications, the replacement looks evident and valuable, presenting huge business opportunities and benefits for a more sustainable production and economy. Bio-based materials showed a global market capacity of 2.4 million tons in 2021, forecasting an increase to 7.6 million tons by 2026 (European Bioplastics 2021). Packaging represents the greatest segment, with a 48% global production capacity in 2021. This scenario has also boosted the demand for biopolymers over the last years regarding technological innovations and commercial applications.

Biopolymers can be obtained from natural sources (starch, cellulose, alginate, chitosan, gelatin), biosynthesized by microorganisms (polyhydroxyalkanoate – PHA, polyhydroxybutyrate, PHB, bacterial cellulose), or chemically synthesized from agro-resources (polylactic acid, PLA). They are derived from renewable marine and agricultural resources and/or food processing wastes and by-products, and also natural fibers, which can provide reinforcement properties to the materials. Biopolymers and their combinations offer many possibilities and opportunities for various industries/sectors, including food packaging, paper, agriculture, medical, textile, and automotive (George et al. 2020).

Synthetic polymers have been extensively used over the years due to their easy processability, low price, high chemical and mechanical stress resistance, and great versatility. These molecules can be designed in a wide variety of physical and chemical characteristics by adjusting their molecular weight, chemical functions, and processing parameters. Biopolymers materials, such as films and coatings, usually exhibit lower mechanical properties and stability when compared to synthetic ones. To overcome such limitations and increase competitiveness, composite materials can be obtained by combining multiple biopolymers and/or micro/nano reinforcement components. These composites, which can display different phases, take advantage of pure materials' characteristics to attain enhanced structures. They can be classified into three categories: composites containing suspended reinforcing particles; multilayered structures of two different materials usually exhibiting strong physicochemical interaction between layers; and fiber-reinforced composites (George et al. 2020). The use of biobased nanoparticles and nanofibers usually impacts the mechanical properties, heat deformation temperature, gas permeability, and decomposition of the resulting biopolymer film. Blending different biopolymers to form films or coatings usually results in a material whose properties are expected to be a combination of those of the biopolymers alone.

Biopolymer films and composite coatings have various application possibilities in many different areas, boosting the interest of researchers and producers in developing novel alternative biodegradable materials. In this chapter, the relevance and recent advances in biopolymers films and composite coatings will be discussed, highlighting their applications in different sectors such as food packaging, medicine, textile, and agriculture.

Biopolymer Films and Coatings Preparation Methods

Despite sometimes being interpreted as synonyms, films and coatings present distinct producing processes. Films are produced as pre-formed structures, which are then applied over the product surface or between its components. On the other hand, coatings are formed by the direct application of the film-forming solution on the product surface (Falguera et al. 2011).

Biopolymer films can be formed by solvent casting (wet process) and extrusion (dry process). Casting is the most convenient method for producing biopolymer-based films. It consists in dissolving the biopolymer in an appropriate solvent (water

or other organic solvents), which may include additives and fillers addition, heating, and pH adjustment, and then pouring the solution directly into the support, followed by drying, forming a thin layer, usually 20–200 μm thick. The film thickness can vary with volume applied, solid concentration, final moisture content, and swelling characteristics (Bierhalz et al. 2020). Extrusion technology has gained much attention for the large-scale production of bio-based films since it can provide high productivity and use conventional plastic industry equipment. It uses thermoplastic biopolymers associated with plasticizers and low moisture levels and can be used for the large-scale production of films (Rajeswari et al. 2021).

Biopolymer coatings can be prepared by dip-coating, spread-coating, and spray-coating, and the chosen method determines the coverage efficiency and the cost of the process. The performance is also dependent on the selection of the coating solution (type, concentration, viscosity, and surface tension) (Bierhalz et al. 2020). Dip-coating involves dipping the product in the biopolymeric solution and then removing it at a constant rate, followed by solvent evaporation by drying. Layer-by-layer (LbL) technique is a variation of the dip-coating in which bi- or multilayered systems are formed by sequential application of the biopolymeric solutions. The thin layers formed are bonded together by physical or chemical interactions. In spread-coating, the coating dispersion is applied directly on the food material or packaging surface by using spreaders, brushes, rods, or spatulas. Spray-coating can be done using classical spraying methods, such as compressed air-assisted sprayer and knapsack sprayer, as well as electro-spraying method. One of the main advantages of spray coating is the possibility to be used on large and non-regular surfaces. A semi-permeable membrane is formed on the products by distributing the coating solution over the targeted surface through the formation of droplets. The droplet sizes usually reach about 20 μm for conventional spraying methods, while electro-spinning can result in much smaller sizes, around 100 nm (Tavassoli-Kafrani et al. 2016). Bio-based coatings technologies are promising for obtaining antimicrobial, hydrophobic, and self-cleaning surfaces, and numerous sectors can benefit from those technologies, such as the food, textile, pharmaceutical, agriculture, and paper and packaging industries. The biopolymer in the solution must constitute a sufficiently cohesive structural matrix. Other components can be added to the coating solution to enhance coating characteristics. Lipids and surfactants can be used to reduce hydrophilicity and water vapor permeability. In contrast, plasticizers, such as polyols and sucrose, can reduce the glass transition temperature and improve the flexibility of the polymeric matrix.

Food Packaging Applications

The increasing consumer demand for a diverse and constant offer of fresh produce and innovative and convenient food products has boosted the search for new food packaging solutions. Packaging that can maintain product quality and safety while minimizing environmental impacts associated with conventional plastic disposal imposes a great challenge to researchers and industries. Primarily, food packaging is intended to protect food from the external environment by avoiding or reducing

contact with microorganisms, exposure to oxygen, temperature, and humidity, therefore promoting the extension of the shelf-life. Other important features include communication, marketing, and convenience of use and storage (Kumar et al. 2020).

The relatively low cost, lightweight, versatility, and good barrier and mechanical properties make conventional plastics the most popular option for packaging food products. However, the intensification of consumers' concerns about environmental and health issues has brought focus on alternative bio-based food packaging materials. Such bio-based alternatives include as potential sources to produce edible films and coatings, mainly polysaccharides (e.g., cellulose, starch, chitosan, alginate, pectin, and agar) and proteins (casein, collagen, soy protein, whey protein, gluten, and zein). Besides biodegradability, biopolymeric films and coatings must ensure food safety, and mechanical, thermal, and barrier properties. The great diversity in chemical structure and functional groups provides possibilities for enhancing or tuning bio-based materials' properties by chemical and/or physical strategies. These include chemical modifications (esterification, grafting, and cross-linking), the addition of plasticizers, mixing with other biopolymers, nanofillers, and other substances that promote better miscibility (Garavand et al. 2017). Protein and polysaccharide films intended for food packaging usually exhibit higher performance through cross-linking the polymeric matrix. The main impact of cross-linking is the decrease in water sensitivity, one of the major drawbacks of biopolymers films and coatings causing swelling and disintegration. It also improves thermal, optical, and chemical stability (Azeredo and Waldron 2016). Cross-linking can be chemical or physical, involving the linking of the polymer chains by covalent or non-covalent bonds, respectively, forming tridimensional networks. Chemical cross-linking with some aldehydes (e.g., formaldehyde and glutaraldehyde) are very effective, especially with protein, but have been avoided due to the possibility of aldehyde residue migration. Calcium-induced physical cross-linking of polysaccharides results mainly from electrostatic interactions between calcium ions and uronate blocks in alginates and pectins chains, known as the "egg-box" model (Bierhalz et al. 2022). For food applications, it is essential to consider that the formation process of films and coatings must assure food contact requirements, such as pH, use of food-grade ingredients and solvents, enzymatic reactions, and thermal treatment.

The properties of films and coatings can be manipulated based on the characteristics of the biopolymers. Blending two or more carbohydrates and/or proteins is a common approach to improve matrix properties. Hydrophobic molecules, such as lipids, demonstrate strong moisture barrier properties, whereas hydrophilic molecules, such as polysaccharides and proteins, demonstrate efficient mechanical properties and good gas barriers such as oxygen and carbon dioxide. Such combinations can be achieved by the complexation of a hydrophilic layer with a hydrophobic layer or emulsion-based films by evenly distributing the hydrophobic component into the polymeric matrix.

Another important feature of biopolymeric films and coatings is their ability to act as a vehicle and promote the controlled release of active agents (antioxidants, antimicrobials, enzymes, probiotics, prebiotics, vitamins) that can act on the surface or be incorporated into the food bulk, improving quality and shelf-life (Kumar et al. 2020).

Fruits and Vegetables

Consumers' growing preference for a healthy and nutritious diet has driven demand for fresh fruits and vegetables free of pesticide residues and extended shelf-life. However, these products are highly perishable, and maintaining the post-harvest is complex. Quality loss occurs during harvesting, storage, and distribution until reaching the consumer, especially when long distances are involved. Losses along the supply chain are estimated at around one-third of world production (Gustavsson et al. 2011). Tropical fruit losses, for example, are estimated to be about 18–28% after harvest (Md Nor and Ding 2020). Among the main factors that affect the quality and, consequently, the shelf life of fruits and vegetables are biochemical modifications, post-harvest microbial deterioration, and physical damages during handling and transportation (Basumatary et al. 2022). Therefore, reducing losses and waste increases food availability for an ever-increasing population and reduces pressure on natural resources.

Fruits and vegetables are living tissues with high moisture content (60% to 95%) and water activity. After harvest, the respiration process continues using food reserves to produce carbon dioxide, heat, and water. Water is also lost to the surrounding atmosphere through the process of transpiration. These natural metabolic processes lead the fruit to senescence, with initial shrinkage and subsequent weight loss and deterioration of appearance (Flores-López et al. 2016). Chemical and biochemical processes; non-enzymatic browning; oxidation of chlorophyll, anthocyanin, pigments, and vitamins; and the conversion of starch to sugar are responsible for accelerating color deterioration, off-flavor, and loss of nutritive value of the fruits and vegetables (Basumatary et al. 2022). In addition, due to the high moisture content, low pH, and nutritional composition, fruits and vegetables are susceptible to microbiological damage. These deterioration processes have motivated the food and horticulture industry and researchers to obtain methods to maintain the quality, food safety, and freshness of these products. Films and coatings based on biopolymers have become prominent strategies for improving the shelf-life of several fruits and vegetables, such as apples, cherries, grapefruit, cucumber, strawberry, tomato, and capsicum.

Fruit and vegetable coatings change the atmosphere in the headspace, increasing the CO₂ concentration and reducing the O₂ concentration. This environment favors reducing respiration rate and conserving stored energy, prolonging the shelf life. The semi-permeable coating characteristic restricts the exchange of gases and water vapor but does not create an anaerobic environment that causes a loss of fruit quality. Therefore, barriers to water vapor and gas are the fundamental functional properties of edible films and coatings. It is also essential that edible films and coatings have a minimal impact on the color and gloss of the products and appropriate mechanical strength to prevent their tearing and protect the products from physical damage.

In addition to reducing moisture loss, gas exchange, respiration, and oxidative reaction, edible coatings are also suitable for incorporating several active

compounds, including antimicrobials, antioxidants, flavors, dyes, sweeteners, and vitamins, increasing the sensory attractiveness, and improving nutritional values. Edible films can also prevent fruits and vegetables from chilling injuries and storage disorders.

The components of edible coatings must be classified and recognized as GRAS (recognized as safe) because they are consumed with the product (Flores-López et al. 2016). Thus, polysaccharides and proteins have been extensively evaluated to produce films and edible coatings, used individually and together. The choice of coating material depends on the desired properties, as each polymer has unique characteristics. In general, polysaccharides are hydrophilic and result in coatings with poor moisture barrier but satisfactory mechanical properties. On the other hand, proteins have the desirable advantage of gas barrier properties without anaerobic conditions but also present a minimal moisture barrier (Md Nor and Ding 2020).

A wide variety of polysaccharides can be used as coatings for fruits and vegetables with proper adhesion, gas barrier, and mechanical properties. The most common are chitosan, starch, alginate, pectin, cellulose derivatives, carrageenan, and gums (Arabic gum, agar, guar, xanthan). As polysaccharides coatings have a poor moisture barrier, to delay moisture loss from fruits and vegetables, they can act as a sacrificial agent, in which the coating is dehydrated instead of the food.

Chitosan is widely used as coatings for fruits and vegetables because it lacks toxicity, biodegradability, excellent film-forming ability, gas barrier properties, and antimicrobial and antioxidant activity. In addition, chitosan is compatible with several compounds, such as vitamins, minerals, and antimicrobial agents, maintaining its nutritional and qualitative characteristics. Many studies support the potential role of chitosan coating in prolonging the storage life and controlling the decay of fruits and vegetables, such as papaya, apple, peach, broccoli, tomato, mango, carrot, and banana. The attributes of chitosan coatings for fruits and vegetables also include preventing the decrease in the content of natural antioxidants during post-harvest storage, delaying browning and suppressing fungal diseases, and inducing the production of enzymes that act in defense of plants, such as chitinase (Dhall 2013).

Antimicrobial activity is one of the most attractive properties of chitosan and depends on several factors, such as deacetylation degree, molecular weight, and concentration at which it is applied. Antimicrobial activity plays a significant role in the control of postharvest decay, accounting for 35–45% of coating effectiveness (Romanazzi et al. 2017).

Solubility, antimicrobial activity, and interaction capacity can be improved with chitosan in nano form. Chitosan nanoparticles exhibited higher antimicrobial activity due to the small and compact particle size and high surface charge. Divya et al. (2018) prepared chitosan nanoparticles by ionic gelation and confirmed its ability as a coating of material to improve the shelf life of tomato, chilly, and brinjal. For instance, the weight loss of tomato coated with 5% chitosan nanoparticles after 5 days of storage was 0.21%, while tomato coated with chitosan

presented a weight loss of 9.33%. For tomatoes without coating, the weight loss was 25%.

In addition to controlling postharvest decay of fruits and vegetables, chitosan has also been used to prevent plant diseases. Some examples of commercial formulations include Chitoplant (ChiPro GmbH, Bremen, Germany), Biochikol 020 PC (Gumitex, Lowics, Poland), Armour-Zen (Botry-Zen Limited, Dunedin, New Zealand); Biorend (Bioagro S.A., Chile), and Kendal Cops (Valagro, Atesa, Italy).

Cellulose derivatives such as methylcellulose (MC), carboxymethylcellulose (CMC), hydroxypropyl cellulose (HPC), and hydroxypropyl methylcellulose (HPMC) are also attractive for use as edible films and coatings for a variety of fruits and vegetables. They impart moisture and gas barriers, good adhesion to the food surface, retain the firmness and crispness, and can preserve flavor components (Nieto 2009). Commercial formulations based on CMC include Semperfresh™ (AgriCoat Industries Ltd., Berkshire, UK), composed of sucrose esters of fatty acids, sodium carboxymethylcellulose, mono-diglycerides of fatty acids, recommended for pear, melon, avocado, apple, cherry, plum, banana, and lime.

The use of alginate as an edible coating is closely related to its ability to form translucent, shiny, strong, and insoluble cross-linked structures with divalent ions such as calcium. An interesting aspect is that calcium chloride is a texture-firming agent since it can form complexes with low methoxyl pectin present in fruits and vegetables. Therefore, a similar benefit can be obtained with sodium alginate-calcium chloride (Alharaty and Ramaswamy 2020). Alginate- Ca^{2+} coatings have been used successfully to prolong the shelf life of fresh-cut Gala apples without causing fermentation due to undesirable anaerobic respiration. Applying calcium-alginate coatings on fresh-cut Gala apples has been demonstrated to reduce weight loss, browning, and mold growth and preserve firmness during storage (Alharaty and Ramaswamy 2020). This polymer has also been explored in fresh-cut fruits with high moisture surfaces, such as strawberries, melon, and watermelon.

Proteins such as gelatin, corn zein, casein, keratin, soy protein, wheat gluten, and collagen have been used for edible coatings for fruits and vegetables. Due to their hydrophilic nature, protein-based coatings are susceptible to moisture absorption, being affected by humidity and temperature. Incorporating fatty acids or using cross-linking agents can improve the water vapor permeability of protein-based films and coatings, but their edibility can be compromised (Dhall 2013).

Zein is one of the most explored proteins used as a coating in fruits and vegetables. This protein contains many hydrophobic amino acid residues, providing zein films with low water vapor permeability compared to most other proteins and good gas barrier properties. The benefits of zein coatings have been demonstrated on harvested fruits and vegetables such as pear, mango, apples, apricot, guava, and tomato.

Matrix formulations composed of polysaccharide-polysaccharide, protein-protein, and polysaccharide-protein have become an economical and versatile way to obtain new materials with better properties. The common objective of blends is to get material with better performance than individual polymers. The preparation of

films and coatings consisting of two or more biopolymers for application in fruits and vegetables is widely reported in the literature (Basumatary et al. 2022).

Nanomaterials such as nano-ZnO, nano-TiO₂, nano-silica, nanosilver, cellulose nanocrystals, and montmorillonite, owned to large surface area and high surface energy, are usually incorporated into biopolymers matrices to improve several properties, such as gas and water vapor barrier, thermal stability, and mechanical resistance.

Polysaccharide-ZnO nanocomposites, besides improving mechanical and barrier properties related to weight loss, respiration, and oxidation, also contribute to the inhibition of microbial growth of fruits and vegetables (Anugrah et al. 2020).

TiO₂ has been reported to exhibit antimicrobial and ethylene photodegradation activity when exposed to UV light by generating hydroxyl radicals and reactive oxygen species (ROS) on its surface, reacting with organic molecules. Nano-composite coatings of this nanoparticle in the chitosan matrix can delay the ripening process of tomatoes (Kaewklin et al. 2018) and reduce losses caused by decay and water dispersion of mangoes (Xing et al. 2020).

Nano-silver is widely used in films and coatings due to its antibacterial effect against 150 types of microbes. In a study on the preservation of quality of shiitake mushroom, alginate/nano-silver coating efficiently reduced the growth of mesophilic, psychophilic, pseudomonas, yeast, and molds and inhibited weight loss, softening, and browning after 16-days of storage (Jiang et al. 2013).

Non-climacteric fruits such as citrus fruits commonly receive lipid-based coatings (e.g., waxes and resins) to provide an attractive appearance, while reducing CO₂, O₂, and water vapor permeability. However, lipid-based coatings may encourage the accumulation of CO₂ and ethanol, causing the development of off-flavors (Flores-López et al. 2016). In this case, the association with polysaccharides provides appropriate gas and moisture barrier and mechanical strength.

Essential oils (EOs) are natural compounds obtained from plant materials and considered suitable natural preservatives due to their antimicrobial and antioxidant properties. Although they are generally recognized as safe (GRAS), the direct application of EOs to the surface of fruits and vegetables tends to be avoided due to the influence on the original flavor. Therefore, incorporating EOs into the polymeric matrix can reduce their impact on the flavor and prolong the action time of EOs through the slow-release effect. In addition, EOs can also improve the water vapor permeability of the films, which consequently influence properties such as weight loss, total soluble solids, acidity, and firmness (Yousuf et al. 2021).

The properties and shelf-life of fruits such as apple, peach, guava, mango, grape, banana, tomato, papaya, cucumber, cantaloupe, raspberry, and strawberry have already been evaluated with films and biopolymer coatings containing EOs. Oils from citrus sources (orange, lemon, lime, bergamot, grapefruit), cinnamon bark, and lemongrass are among the most common EOs used in coatings for fresh-cut fruits and vegetables.

The incorporation of active ingredients in films and coatings is considered a strategy of great interest to maintain the quality of fruits and vegetables. Antimicrobials, antioxidants, and anti-browning stand out among the primary agents used.

Enzymatic browning reactions negatively affect the appearance of products, especially fresh-cut fruits and vegetables, reducing consumer acceptability. Organic acids such as ascorbic acid, citric acid, oxalic acid, phenolic acids, and thio-containing compounds are often used to control excessive browning associated with edible films and coatings for fruits and vegetables. Coatings can also encapsulate aroma compounds, flavorings, colorings, and sweeteners enhancing sensory attributes. Functional coatings containing nutraceuticals like minerals, vitamins, and essential fatty acids can improve the nutritional value of fruits and vegetables. However, they may impart a bitter taste, and little information is available regarding the impact on mechanical and barrier properties (Panahirad et al. 2021).

Dairy Products

Dairy products are obtained by processing milk, which may contain food additives and other ingredients needed for processing. Within this category, cheese is one of the products in which edible films and coatings are applied to prevent quality losses. Many physicochemical, biochemical, and microbiological processes occur during cheese fabrication, so choosing the proper packaging can be challenging. Most degradation reactions are accelerated by light exposure and high moisture, such as lipolysis, proteolysis, and lipid oxidation, so packaging must provide low transparency and oxygen and water vapor permeabilities (Chen et al. 2019). Biodegradable films and coatings made from polysaccharides and proteins provide water vapor, oxygen, and carbon dioxide barrier, avoiding weight, flavor, and texture losses (Jafarzadeh et al. 2021). Kampf and Nussinovitch (2000) studied the physical and sensory properties of semi-hard and white-brined cheeses coated with alginate, gellan, and κ -carrageenan solutions. The most promising result was obtained by the κ -carrageenan coating, resulting in improved color and gloss and reduced weight loss along 46 days of storage, conferring a softer and less brittle texture. The barrier properties of packaging materials significantly impact the shelf life of cheese.

Furthermore, active components, such as antimicrobials and antioxidants, can be incorporated into edible films and coatings, hindering the release rates of such substances into the product surface, where microbial deterioration is more prone to take place and, therefore, the concentration of additives in the bulk can be reduced (Bierhalz et al. 2022; Jafarzadeh et al. 2021). Most studies concerning the use of edible films and coatings to protect cheese products include antimicrobial substances as a practical approach to inhibit deteriorating and pathogenic microbial growth. Cheese deteriorates during handling, ripening, and storage resulting in sensory and economic damages (Küçük et al. 2020). Fungi are a major cause of cheese spoilage, and besides quality impacts, some species produce compromising food safety.

Films based on alginate and/or pectin can exhibit distinguished properties by tuning the cross-linking and plasticization degrees (da Silva et al. 2009). Alginate films added with natamycin intended for cheese packaging displayed low O₂ permeability and adequate mechanical and moisture resistance (Bierhalz et al. 2014). Natamycin is a natural bacteriocin, Generally Recognized as Safe (GRAS), allowed

for use in different cheese and meat products. It has limited water solubility, so its addition to a biopolymeric matrix (film or coating) can guarantee a better distribution and antimicrobial action over the product surface (Santonicola et al. 2017). In vitro tests indicated the protective effect of alginate films containing natamycin against fungi species that usually contaminate cheese (Bierhalz et al. 2014). In recent research, Bierhalz et al. (2022) evaluated the effect of natamycin-loaded and natamycin-free alginate films applied over sliced cheese samples contaminated with *Aspergillus niger* and *Penicillium roqueforti* storage at 25 °C for 7 days. The samples covered with the active film showed a significantly lower microbial count (1 log cycle) than the control samples.

Chitosan films and coatings have shown great potential for application in cheese and other food preservation mainly due to the inherent antimicrobial properties associated with its cationic nature. Fajardo et al. (2010) found significantly lower mold and yeast counts for samples of Saloio cheese stored at 4 °C/27 days packed with chitosan films containing natamycin compared to control samples. Moreira et al. (2011) evaluated the antimicrobial effect of chitosan and chitosan/sodium caseinate coatings and films on cheese. They observed a reduced count of mesophilic, psychrotrophic, and yeast and mold in the range of 2 to 4.5 log CFU/g. Chitosan and nano clay nanocomposite exhibit antimicrobial action against spoilage and pathogenic microorganisms on Gouda cheese (Mohammadzadeh-Vazifeh et al. 2020). The shelf life of high-moisture mozzarella cheese was doubled in samples coated with carboxymethyl cellulose with and without natamycin stored at 8 °C compared to uncoated samples (Azhdari and Moradi 2022).

Protein-based films and coatings usually show lower oxygen and water-vapor permeabilities than polysaccharides. This trend can be attributed to the more polar and linear structure, reducing matrix-free volume. A whey protein isolate film was prepared with ginger EO and applied to Kashar cheese. After 30 days at 4 °C, active films showed appropriate water barrier properties and antimicrobial effects against *Escherichia coli* O157:H7 and *Staphylococcus aureus* (Kavas et al. 2016).

Polysaccharide-protein blends are increasingly used in cheese and other various food as they can enhance the functional properties of the proteins and polysaccharides alone. Positively charged chitosan can interact and form three-dimensional networks with opposite charges in proteins, such as caseinate. Antimicrobial films consisting of chitosan and blends of sodium caseinate/chitosan applied either by coating (dipping) the cheese sample with the film-forming solutions or wrapping with pre-formed films hindered mesophilic bacteria, psychrotrophic, yeasts, and molds growth. Sodium caseinate/chitosan films exhibited improved mechanical properties (tensile and impact strengths) and lower equilibrium moisture content compared to chitosan and caseinate neat films due to the strong electrostatic interactions between the two macromolecules (Pereda et al. 2014).

Fresh Kashar cheese samples inoculated with *Listeria monocytogenes* were covered with zein and zein/wax composite films containing natural antimicrobial agent (lysozyme) and natural antioxidants (gallic acid and catechin), and all films were able to inhibit bacterial growth for 8 weeks at 4 °C. However, zein/wax film

reduced the initial microbial load. Gallic acid and catechin in films proved to inhibit oxidative changes in the cheese samples (Ünalán et al. 2013).

Incorporating metal nanoparticles, such as zinc oxide (ZnO) nanoparticles (NPs) in biopolymeric films has been proposed as antimicrobial and photocatalytic systems. Carboxymethyl cellulose and chitosan films containing ZnO-NPs were applied on soft white cheese resulting in prolonged shelf-life (Youssef et al. 2016). For food packaging applications, migration and safety concerning the use of metal nanoparticles still need further investigation.

Eggs

Eggs are a source of high-quality protein, vitamins, and minerals. Its structural components include the shell and shell membranes, the albumen, and the yolk (Almeida e Silva et al. 2020). Several quality problems associated with storing fresh eggs, such as weight loss and changes in egg white, yolk, and pH, can significantly impact the sector. The use of biopolymeric coatings has been proposed to preserve quality parameters and extend the egg's shelf life. Xie et al. (2002) showed that coating eggshells with different biopolymers (soy and whey protein isolates, carboxymethyl cellulose, or wheat gluten) decreased eggshell breakage when compared to control (uncoated) samples. A coating solution composed of methylcellulose and hydroxypropyl methylcellulose added with polyethylene glycol-400 and a mixture of fatty acids was highly efficient in preventing egg quality deterioration stored under ambient conditions extending the shelf life of eggs by 4 weeks. The coating solution formulation was optimized regarding water vapor permeability and mechanical properties, and the lipid component, due to its hydrophobic nature, provided an extra moisture barrier to the coating. The moisture transfer across the eggshell determines the weight loss and albumen changes, so controlling the water vapor barrier of the biopolymeric film is extremely important (Suppakul et al. 2010). In a recent study, Rachtanapun et al. (2021) developed a novel egg-coating material composed of chitosan, carboxymethyl cellulose, and paraffin that successfully covered the egg surface, prolonging the shelf-life and quality parameters by 4 weeks at ambient temperature. The incorporation of paraffin was improved by blending carboxymethyl cellulose with chitosan, thus improving the mechanical properties of the coating. Paraffin acted as a plasticizer and hydrophobic component, enhancing coating flexibility and water resistance.

Concerning food safety issues, the egg production chain requires microbiological control since microfractures in the eggshell can lead to egg contamination by *Salmonella* and other bacteria. Salmonellosis is the most frequent foodborne disease, and eggs have been pointed as the main source of human contamination with *Salmonella* spp. in outbreaks. So, the sanitary quality of commercial eggs represents a bottleneck issue that relies on sanitizing procedures to ensure product safety over the whole production chain (Almeida e Silva et al. 2020). Different chemical compounds have been proposed to avoid contamination, e.g., washing with chlorine-based compounds, such as sodium hypochlorite solution. Due to the

possible toxic effects of chlorine solutions, quaternary ammonium salt components (QACs) are an alternative since they are bioactive surfactants with a broad activity spectrum for different microorganisms. However, the sanitizing effect depends on the chemical nature and concentration of the active agent, but also on the contact time with the product surface during storage. Eggshell coating with chitosan solution containing quaternary ammonium hindered the formation of *Salmonella Enteritidis* biofilm. The coating solution applied by dipping showed reduced surface tension and detergency, promoting an adequate physical and microbiological barrier, protecting eggs, and preventing moisture loss (Almeida e Silva et al. 2020).

Plasticizers are frequently required to enhance film flexibility and improve the mechanical resistance of brittle biopolymers. Besides, the preparation method also influences the efficiency of the coating. Chitosan coatings plasticized with glycerol, propylene glycol, and sorbitol were applied on eggshells by brushing, dipping, and spraying. The shelf-life of all chitosan treated was increased by about 20 days under ambient temperature and better internal quality parameters were obtained by samples coated by brushing and dipping (Kim et al. 2008).

Meat

The meat industry can suffer significant economic impacts from problems associated with lipid oxidation and microbial contamination during product processing, transportation, and commercialization (Domínguez et al. 2018). Pathogenic microorganisms such as *Salmonella Typhimurium*, *L. monocytogenes*, *E. coli*, and *Bacillus* spp. can easily contaminate processing and packaging lines. Biopolymer-based films and coatings can help prevent moisture loss and microbial growth, hinder the oxidation rate, and avoid flavor loss. The coating would act as a barrier to water and gases (CO_2 and O_2), thereby reducing weight loss, color deterioration, aroma deterioration, and maintaining quality (Falguera et al. 2011). So, the meat, pork, and poultry industries could surely benefit from using biopolymers films and coatings for fresh, frozen, and processed meat products.

Films of sodium alginate and agar, plasticized with glycerol, were produced by casting and tested on sliced salami samples stored for 3 months under refrigeration. Samples showed less variation in color compared to control (with conventional packaging) and higher microbiological quality, however, moisture loss was higher in samples wrapped by the bio-based films than in traditional packaging (Puscaselu et al. 2021). Three biopolymeric coatings based on gelatin, turmeric starch, and their blend were applied on fresh frankfurter sausages. All coatings exhibit increased antimicrobial activity over 30 days of storage and moderate mechanical strength. No changes were observed in product color, pH, and texture compared to uncoated samples (Tosati et al. 2017).

Reducing oxygen in contact with the meat product by vacuum or nitrogen packaging is among the most common strategies to prevent lipid oxidation. However, the maintenance of the red color of fresh meat requires high O_2 levels in the packaging. So, lipid oxidation can be delayed by active packaging containing

synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). However, the current demand for safe and more natural products highlights the growing interest in using natural antioxidants.

A great variety of plant extracts and EOs have been used as natural antioxidants for meat and other food products. They are incorporated into the biopolymeric matrix keeping a high concentration of active substances on the surface of the meat. However, the release kinetics of the active agent from the film is a crucial factor to be considered in choosing the biopolymer/antioxidant system (Gagaoua et al. 2021).

Whey protein films containing a mixture of cinnamon and rosemary EOs (2–5%) exhibit a great reduction in the lipid oxidation of pork salami samples (Ribeiro-Santos et al. 2018). Nisa et al. (2015) developed potato starch films with 5% green tea extract for application on fresh beef, while Qin et al. (2013) used chitosan films added with tea polyphenols to hinder the oxidation of cooked pork sausages. In both cases, wrapped meat had lower lipid oxidation ratios than the control and also increased stability of the red color.

In most cases, the incorporation of EO into edible films and coatings has been done through conventional emulsification, but the contact between the EO components and the polymer matrix could be improved with nano-emulsification. Nano-emulsions are a type of emulsion with reduced droplet size (20 to 200 nm) and would, therefore, reduce EO volatilization losses and enhance activity (Basumatary et al. 2022).

The addition of ginger EO in fish skin gelatin films containing ZnO-NPs caused an increase in the elongation and thickness and a decrease in tensile strength and moisture barrier of films. The active films showed antioxidant activity and reduced levels of total volatile bases nitrogen during fresh meat storage. Besides, the films exhibit strong antibacterial activity against food spoilage and pathogenic bacteria (Liu et al. 2020).

Seafood

Fish and fishery products are considered rich sources of protein, unsaturated fatty acids, vitamins, and minerals, and their consumption has increased in recent years. However, fresh fish can undergo fast spoilage because of its high moisture and solute concentrations, weak connective tissue, and neutral pH values, providing ideal conditions for microbial contamination and biochemical oxidation. In fish, lipidic oxidation leads to the formation of bad odors and flavors, harmful molecules, and color changes. Preservation methods like freezing, chilling, vacuum and modified atmosphere, salting, and drying are used in the fish processing industry but still do not guarantee complete microbial and lipid oxidation reactions. So synthetic preservatives such as phosphates, benzoates, nitrite, BHA, and BHT can help lipid oxidation, microbial contamination, texture, and color changes (Hussain et al. 2021).

Following the same trend of other food segments, there is a fast-growing interest in using natural plant and fruit extracts and EOs, bacteriocins, lactoperoxidase, and

bioactive peptides as antimicrobials and antioxidants for fish and seafood (Hussain et al. 2021). These natural components can be vehiculated by incorporating them into polysaccharides and/or proteins films and coatings. Coating solutions can be applied by dipping or spraying onto the fish product surface, enhancing the quality and consumer acceptability. Gelatin and its blends, due to their excellent film-forming properties, have been widely used in fishery products (Abelti et al. 2022).

Chitosan is one of the most promising biopolymers for producing films and coatings for fresh and frozen fish and seafood applications, as it possesses inherently antibacterial, antifungal, and antioxidant activities. The chitosan-free amine groups (NH_3^+) can interact with the negative charges of cell membranes of bacteria, causing microbial inactivation. Other polysaccharides used in fish packaging include cellulose, pectin, alginate, and κ -carrageenan. The antimicrobial effect of a composite coating of chitosan and fish skin gelatin on cod burgers was investigated. Microbial counts of the coated samples were 2 log cycles lower than uncoated ones (López-Caballero et al. 2005).

Biomedical Applications

Biopolymers have advantages that make them attractive for biomedical applications, with emphasis on biocompatibility, biodegradability, non-toxicity, and the possibility of chemical modification, which is highly beneficial for interactions between biomaterials and tissues. Biopolymer coatings are of special interest in implants for orthopedics, dentistry, and cardiovascular applications. These are used to modify surface properties and incorporate additional functionality such as the delivery of growth factors, antimicrobial agents, and drugs. Biopolymer-based films have wide applications as wound dressings and orally disintegrated films (ODFs).

Biomedical Coatings

Polymer assembly into coatings can be done using various physical and chemical methods. Physical methods based on the deposition of the biopolymer directly onto the substrate, such as dip-coating and spin coating, are simple and affordable (Nathanael and Oh 2020). Uniform coatings on substrates of varied sizes can be obtained by the dip-coating technique, which consists of immersing the substrate in the solution containing the biopolymer followed by removal to dry the coating by evaporation. In this technique, the coating thickness depends on several factors: immersion duration, sample removal speed, concentration and viscosity of the polymeric solution, and the number of coating cycles. The spin coating technique can also be used for flat substrates to obtain thin and uniform coatings. In this approach, the solution containing biopolymer is applied in the center of the substrate, which is then subjected to a high rotation speed. In this case, the main parameters are the rotation speed, surface tension, and solution viscosity. Due to the difficulty of rotating at high rates, this technique is more challenging to perform on large

substrates (Song et al. 2020). Other physical methods, including LBL, Langmuir-Blodgett (LB), and plasma-based coating methods, are more suitable for preparing very thin (up to monomolecular) layers of biopolymers.

Despite being straightforward and widely used, physical coatings may have poor stability against mechanical stress, representing a disadvantage in applications such as stents, artificial joints, or catheters. Coatings resulting from chemical bonds between the biopolymer and the substrate are particularly interesting in these cases. Chemical coatings with biopolymers use grafting-to methods, in which the whole molecule can be attached to the surface by creating suitable binding sites, either on the substrate or on the polymer (or on both) (Song et al. 2020).

Metals and metal alloys are used in applications where mechanical strength is crucial, such as hip implants, knee implants, dental implants, or stents. The most used metallic materials are titanium alloys, stainless steel, and cobalt-chromium alloys. In addition to mechanical performance, surface properties are very important for metallic biomaterials, including good cell adhesion, non-toxicity, corrosion resistance, and bacterial biofilm formation (Aydemir et al. 2021). Despite significant advances, the risks of infection can lead to implant failure and repeated surgical intervention, resulting in enlarged clinical and socioeconomic burdens and impaired quality of life for patients.

Coatings based on biopolymers show potential to address multiple issues related to biomedical properties of implants, such as enhanced wear and corrosion resistance, reduced toxicity, and better anti-microbial properties (Fig. 1). They also contribute to increased osteogenesis, improve their cell adhesion, proliferation, and differentiation performances, and carry desirable compounds on their surfaces. Metallic substrates have received coatings of several biopolymers, isolated or in combination, such as collagen, chitosan, alginate, and alginate-collagen.

Chitosan-based coatings on implant surfaces have been reported to enhance protein adsorption, cell adhesion, proliferation, bioactivity, mineralization, and corrosion resistance by incorporating inorganic particles. For dental implants, chitosan coatings can affect mechanical properties at the bone interface, such as elastic modulus, which reduces the mismatch between the implant surface and alveolar bone, reducing stress concentration areas. In an *in vitro* study of dental implants coated with chitosan, a high deacetylation degree of chitosan was shown to increase biomineralization and osteoblast cell formation (Alnufaiy et al. 2020).

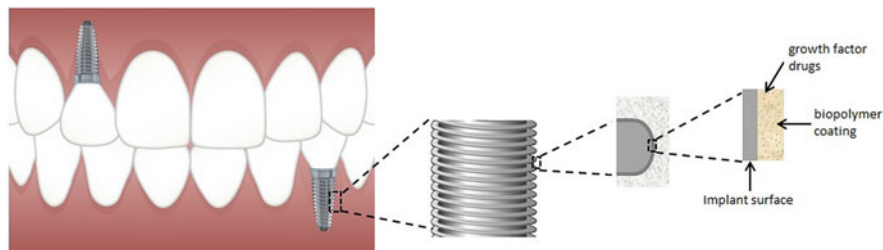


Fig. 1 Biopolymer coating on dental metallic implant

Efforts have been directed toward increasing the corrosion resistance of magnesium alloys. These alloys are attractive because they are degraded autonomously in the biological environment, which avoids the second surgery in the case of fractures. However, they are susceptible to corrosion in the physiological environment, whose degradation products can damage cells. Multifunctional calcium phosphate and chitosan coatings can improve corrosion resistance, cytocompatibility, and antibacterial ability.

Collagen coatings have been proven beneficial for osteoblast activity, implant stability, and osseointegration, facilitating the mineralization of the extracellular matrix and the expressions of specific osteogenic markers. A study involving the implantation of titanium orthopedic screws coated with chitosan into the femurs of New Zealand white rabbits demonstrated that collagen coating enhanced bone fixation. The coating also created a more well-anchored and stable implant than the traditional hydroxyapatite coatings (O'Neill et al. 2021).

Duan et al. (2021) reported that the corrosion resistance of titanium implants and crowns used in dentistry is prone to be severely damaged in the presence of aggressive fluoride ions from toothpaste, prophylactic gels, and mouthwashes. These authors developed a chitosan-alginate coating with self-strengthening capability in artificial fluorine-containing saliva. The anticorrosion performance was improved by forming a calcium alginate gel in the presence of calcium ions in the corrosive medium.

Strontium ions can promote osteogenic activity and, as divalent ions can cross-link alginate, coatings of this biopolymer can be used as a delivery platform for sustained release. In this case, the slow release is favored by chelation with sodium alginate (Yuan et al. 2017).

Wound Dressings

The human skin is the largest multifunctional organ of the body and provides protection, thermoregulation, sensation, and immune functions. As a barrier organ, the skin is constantly susceptible to environmental risks, such as chemical, physical, and thermal injury. Skin lesions occur with disruption in the continuity of the epithelial line and can be categorized as acute and chronic according to the duration and nature of the healing process.

Wound dressings play a crucial role in the complex regeneration process of skin lesions. An ideal dressing must satisfy several requirements: provide a moisture environment, remove excess fluids from the wound, reduce pain, prevent microbial contamination, enhance epidermal migration, and promote angiogenesis and connective tissue synthesis. It is also important that the dressing is non-adherent and easy to remove, non-toxic, non-allergic, flexible to fit body contours, and able to deliver therapeutic compounds.

Some properties required for wound dressings are related to the wound characteristics. For example, the swelling capacity of a wound dressing must be high for lesions with high exudate production. Also, the water vapor transmission rate

(WVTR) of films and membranes depends on the type of wound, with minor injuries requiring low WVTR, while wounds with granulation tissue may require rates up to 20 times higher. Regarding mechanical resistance, it is essential to consider whether the injured area requires elasticity, such as joint regions (Bombaldi de Souza et al. 2020).

The use of biopolymers as a material for wound care has a wide scope due to their excellent biocompatibility properties, ability to support cell growth, regenerative potential, biodegradability, and durability. The most used biopolymers for wound healing applications are chitosan, alginate, bacterial cellulose, hyaluronan, collagen, silk fibroin, and gelatin. These biopolymers can be used alone or in combination and may also contain bioactive agents such as antibiotics, antimicrobials, and anti-inflammatories that will improve the therapeutic action. The films intended for wound dressing applications are obtained mainly by casting technique.

Polysaccharides have been extensively used as matrices for wound dressings, especially chitosan, alginate, hyaluronic acid, and bacterial cellulose.

Chitosan presents a hemostatic effect and stimulates macrophage activity, accelerating tissue regeneration. Chitosan and its derivatives play roles in wound healing, mainly in the first three stages. In the first stage, it helps to stop bleeding. In the second phase, called inflammation stage, chitosan contributes to cleaning the bacteria from the wound. In the third stage, chitosan contributes to the growth of granulation tissue and skin proliferation (Feng et al. 2021).

In addition to several studies related to the development of chitosan films and membranes for application as wound dressing, many chitosan-based dressings are commercially available, such as ChitoSorb (ChitoTech), KytoCel (Aspen Medical), Opticell (Medline), and Chitoflex (HemCon).

Alginates and their derivatives have also been explored for skin lesions treatments and reported to have anti-inflammatory effects, regenerative, and angiogenic properties. Alginate membranes cross-linked with calcium ions are attractive for wound healing, exhibiting good drug delivery and barrier properties, hemostatic, and highly absorbent character. As wound exudates are rich in sodium, there is an exchange of these ions with the calcium ions of the cross-linked matrix, resulting in a gel that contributes to maintaining a moist microenvironment. Therefore, the formation of granulation tissue and reepithelization is favored in the healing process. The list of commercially available alginate-based wound dressings is vast and growing fast. Some examples are Algicell (Derma Science), Kaltostat (ConvaTec), Tegaderm (3 M Healthcare), and Algisite M (Smith & Nephew).

Bacterial cellulose (BC) has outstanding properties that make it an important biopolymer for developing improved wound dressings, including severe wound healing. In addition to its porous structure, biodegradability, histocompatibility, and nontoxicity, BC is highly pure and can be used without prior treatments. BC films are easy to remove, reduce inflammation, and have a high capacity to retain moisture, which can vary from 60 to 700 times their dry weight, which aids in the absorption of exudates, prevents the dehydration of wounds, and promotes fast healing. Another advantage of BC is its transparency, which provides heat absorption and pain reduction in cases of burn wounds. The BC structure's microporosity

favors the drug's slow release to the wound. However, it does not impair the ability of the films to act as a physical barrier against external agents (Bombaldi de Souza et al. 2020).

Hyaluronic acid is found as a component of the skin's extracellular matrix (ECM) and is involved in the wound healing process, decreasing the inflammatory cells infiltration, improving the re-epithelization and granulation, as well as increasing the formation of blood vessels that are important for improving skin regeneration. Despite the biological attractiveness, high production costs, the possible presence of contaminants due to the extraction process, the low water stability, and weak mechanical properties have limited the application of hyaluronic acid films. The rapid degradation and high swelling of hyaluronic acid may require periodic dressing replacements and result in low biochemical functionality for cell attachment and proliferation. Therefore, different strategies have been studied to overcome these limitations, such as cross-linking, chemical modification, and/or conjunction with other polymers, such as methylcellulose, chitosan, and collagen. Commercial dressings based on a hyaluronan derivative, a benzyl ester of hyaluronan (HYAFF®), are available. For example, Hyalosafe® is a transparent film dressing indicated for superficial lesions with moderate exudate production, such as first and second-degree burns.

Proteins such as collagen, keratin, and silk fibroin, have promising potential in wound healing. Among these proteins, collagen is particularly interesting as it is the main component of the dermal extracellular matrix, playing a crucial role in the healing process. Dressings based on this protein have a highly absorptive and hydrophilic character and are indicated for many types of wounds, such as chronic wounds. Collagen films, however, have high permeability to bacteria and microorganisms, and daily dressing change is recommended in cases of wound infection. To improve mechanical stability and degradation rate, collagen-based films require cross-linking with chemicals (sorbitol, glycerol, glutaraldehyde, hexamethylene diisocyanate) or biological agents (transglutaminase and k-carrageenan).

Silk fibroin is of increasing interest in wound healing applications. In addition to excellent biocompatibility, this material has high water uptake, good air permeability, slight bactericidal properties, low immunogenicity, hemostatic properties, slow degradation, and remarkable mechanical properties. To induce crystallization and improve stability, films are crystallized in organic solvents such as methanol. However, this treatment makes silk fibroin films brittle in the dry state. The flexibility of the SF films can be improved by blending with other natural polymers or by incorporating plasticizers.

Zhang et al. (2017) performed an in vivo study using silk fibroin films to treat wounds in animals (rabbits and porcine) and humans. Compared with commercial dressings, the silk fibroin film reduced the incidence of adverse events and the average healing time. The films allowed visual observation of dermal wound healing, could be easily applied and removed, and showed excellent barrier and fluid absorption properties.

As the major protein of the outer epidermis, keratin has intrinsic wound healing efficacy involved in all stages of wound healing. Keratin-based dressings

demonstrate to stimulate keratinocyte activity and increase cell adhesion, migration, and proliferation. A clinical study with patients with superficial and partial thickness burns showed that keratin-based healing products facilitate healing with minimal scarring and presented several other advantages, such as ease of use, low cost, and good patient tolerance, with minimal pain and itching (Loan et al. 2016).

Keratin films and membranes are transparent, highly elastic, allow gas exchange and water penetration at the wound area, and prevent bacterial access. Limitations are related to low absorption exudate capacity and mechanical performance. Because of this, keratin is usually combined with other natural and synthetic polymers to create wound dressings. The challenge is to improve mechanical strength without sacrificing biological efficacy.

Proteins of non-animal origin are less common but also demonstrate potential for wound healing. Peles and Zilberman (2012) reported that the non-animal origin of soy protein eliminates the risk of transmissible diseases that pose a danger in products of human and animal origin. In addition, soy protein has good water resistance, long shelf life and stability, and degrades into natural components. These authors produced soy protein films and observed that the physical properties of the wound dressing (water absorbance and weight loss profile) could be controlled by the cross-linking process. Regarding water vapor permeability, the films presented values suitable for wound dressings ($\sim 2300 \text{ g m}^{-2} \text{ day}^{-1}$), and this property was not affected by the cross-linking method.

Combining two or more polymers to obtain blends and composites is a simple approach for tuning the properties of the wound dressings. Chitosan stands out as one of the most studied biopolymers for the formation of blends, which comes from its cationic nature that allows the formation of polyelectrolyte complexes with various anionic polymers such as alginate, pectin, hyaluronic acid, collagen, gelatin, silk fibroin, among others. In general, mechanical properties, moisture resistance, biocompatibility, and control of the release of active agents can be improved.

Regarding composites for wound dressing applications, the increase in mechanical strength and antimicrobial activity are the most desired goals. Therefore, Ag, ZnO, TiO₂, and CuO nanoparticles have been evaluated to form nanocomposite films with biopolymers. Several active compounds, such as antioxidant drugs, antibiotics, anti-inflammatory drugs, natural compounds with therapeutic activities, such as EOs, and growth factors can be incorporated into biopolymer matrices to maximize dressing efficiency.

Orally Disintegrating Films (ODFs)

Administering drugs orally is considered the preferred route by patients (Irfan et al. 2016). The use of biopolymer-based films for drug administration in the oral cavity has recently received increasing attention. These orally disintegrating films (ODFs) offer a precise dose administration and are easy to handle. An important advantage for patients with swallowing difficulties is that the administration of ODFs does not require water ingestion. In addition, drug absorption by the mucosa is a way to

prevent the degradation of active compounds in the gastrointestinal tract (Garcia et al. 2020).

The main requirement for a polymer film to be used as ODF is its rapid disintegration when in contact with saliva, releasing active pharmaceutical agents from the dosage form. Polymers used to produce oral films should be nontoxic, nonirritant, and have good wetting and spreadability. Among the most used are gelatin, pullulan, starch, sodium alginate, pectin, chitosan, maltodextrins, hydroxypropyl methylcellulose (HPMC), sodium carboxymethyl cellulose (CMC), and hydroxypropyl cellulose (HPC). The association of different polymers can contribute to better film properties, such as hydrophilicity, flexibility, mouthfeel, and solubility (Garcia et al. 2020).

The production of ODFs usually uses polymers (40–50% w/w), plasticizing agents (0–20% w/w), and active compounds (1–25% w/w). Other components, such as flavoring agents, sweeteners, and saliva stimulants, may be added in concentrations up to 40% w/w to improve acceptance. ODF properties can be modified by varying the polymer concentration used in the formulation, reaching up to 60–65% w/w. Regarding the methods, the main ones currently used are casting and hot extrusion to produce films having an area ranging from 5 cm² to 20 cm² (Irfan et al. 2016).

ODFs have been developed to treat various medical conditions such as allergies, pain, addiction, sleep difficulties, and Alzheimer's disease. ODFs have also shown potential for aesthetic purposes. For example, Lee et al. (2022) demonstrated that 22 women treated over 12 weeks with ODFs containing collagen had skin wrinkles reduced, and elasticity and density significantly increased.

Textile Applications

Functional textiles are one of the most important fields in textile science and industry. Functionalities can include antimicrobial activity, self-cleaning, antistatic, insect repellency, heat and cold resistance, anti-creasing, flame retardancy, and protection against ultraviolet radiation (Fig. 2). Biopolymers have played a significant role in obtaining these functionalities through coatings on the most diverse types of natural and synthetic substrates.

Well-established methods of textile finishing, such as exhaustion, impregnation, spraying, and LbL deposition, can be explored in the application of biopolymers coatings (Asif and Hasan 2018). Pad-dry-cure is one of the most industrially used methods to produce textile polymeric finishes. The process is accomplished by padding fabric through a biopolymer solution, squeezing between rolls at a pressure to control the pick-up, drying at moderate temperature, and curing at an elevated temperature (Malucelli et al. 2014).

A relevant disadvantage related to biopolymeric coatings is the poor adhesion to the fibers, which can result in gradual leaching with washing processes. Chitosan, for example, forms weak bonds with cellulose fibers based on hydrogen bonds and van der-Waals interactions. Cross-linking agents such as polycarboxylic acids can be

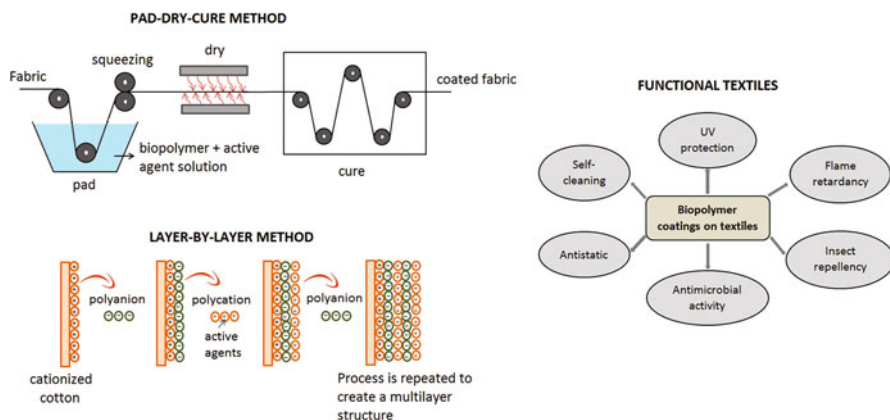


Fig. 2 Representation of pad-dry-cure and layer-by-layer coating methods for the development of functional textiles

used for stronger bonds, resulting in greater treatments' durability. In this case, the amine groups of chitosan and hydroxyl groups of cellulose can form covalent bonds with the carboxyl groups of polycarboxylic acid.

Coatings must not adversely affect the fabrics' feel, handling, and breathability. Therefore, nanocoating, which has thicknesses of less than 1 μm (Joshi et al. 2011), represents a promising alternative to provide functionality while preserving tissue characteristics. The LbL technique has been highlighted for obtaining nanocoating on textile substrates. The substrate surface is exposed to the polymer with a charge opposite to the substrate surface charge for times ranging from seconds to minutes. Multilayers are then constructed by the sequential adsorption of oppositely charged polycations and polyanions. Cotton inherently has an anionic character but is often subjected to modification processes, such as cationization or anionization, for more efficient electrostatic interactions.

Flame Retardancy

Halogenated and phosphorus chemical compounds are very efficient in obtaining textile materials with flame retardant properties. However, the fact that these materials are proven to be toxic has motivated the search for halogen-free substituents. Efficiencies even comparable to conventional phosphorus-based flame retardants can be obtained using biopolymer coatings under mild conditions (aqueous suspensions/solutions) with no volatile organic carbon (VOC) produced and with low human and environmental impacts (Malucelli et al. 2014).

Recently, alginate has emerged as an excellent candidate for eco-friendly and phosphorus-free coating flame-retardant textile. Alginates had inherent flame retardancy due to their ability to cross-link with polyvalent metal ions, such as calcium (Xu et al. 2021). For polyester fabric, Liu et al. (2018) demonstrated that

a coating with calcium-alginate/nano-calcium borate composite can improve flame retardant and anti-dripping properties.

LbL assembly method has been successfully used as a finishing technique to produce flame retardant fabrics by combining, for example, positively charged polyethyleneimine and negatively charged alginate with subsequent cross-linking. When comparing the cross-linking of alginate with barium, nickel, and cobalt ions, Pan et al. (2017) observed that cross-linked barium coating could protect the modified cotton fabric during a burning test resulting in a complete and stable char residue.

LbL technique can also be used to produce intumescent coatings with chitosan and phytic acid. An intumescent system consists of a carbon source, an acid, and a blowing agent. During heating, these components react to form an insulating layer that protects the fabric from flames. In this case, chitosan serves as a char-forming agent and acts as a blowing agent, releasing ammonia gas as it degrades. Swelling systems using chitosan with retardant activity were observed for cotton (Laufer et al. 2012) and polyamide 66 (Kundu et al. 2017). Protein coatings also demonstrate potential for developing textiles with flame retardant properties. The effect of several deposited proteins on the flame retardancy behavior of the surface of textile substrates was investigated, such as whey protein, egg white protein, casein, and soybean.

The use of casein is based on its high phosphorus content. In cellulose substrates, casein may favor dehydration, generating thermally stable carbonaceous structures, rather than the depolymerization of glycosyl units into volatile combustible species capable of further fueling the process. Consequently, the total burning time increases, and the total burning rate decreases, indicating an enhanced flame resistance. Carosio et al. (2014) compared flame retardancy of cotton, polyester, and polyester-cotton fabrics coated with caseins and observed self-extinguishment only for cotton. For polyester and polyester-cotton blends, the burning rate was strongly reduced (70% and 40%, respectively), but the melt dripping phenomena for polyester were not suppressed.

In addition to delaying or preventing thermal degradation, the water adsorption capacity of proteins could partially dissipate the heat during combustion and dilute the produced volatile species. For cotton, for example, whey protein coatings resulted in a long time for the combustion process to take place, but a self-extinguishment of the flame was not achieved (Bosco et al. 2013).

Proteins can also be combined with phytic acid using the LbL technique to form a flame retardant system arising from a synergistic effect of phosphorus (from phosphate groups of acid) and nitrogen (from amino acids from protein) that leads to high residue and time to ignition of fabrics (Liu et al. 2020).

Antimicrobial

Textile materials are susceptible to microbial growth, which can generate unpleasant odors, degrade mechanical properties, cause discoloration, and even dermal

infections in users. Besides aesthetic and hygienic purposes, an emerging segment of textile antimicrobials is medical textiles, which include surgical threads, gauzes, bandages, hospital clothes, and protective clothing (surgical masks, caps, gowns).

Chitosan is the biopolymer that has received the most attention due to its intrinsic antimicrobial action. Even if the main objective is antimicrobial activity, other benefits can be derived from this treatment for cotton fabrics, such as dyeing improvement, abrasion resistance, antistatic, and anti-crease properties. Yellow zein, rich in lutein and zeaxanthin carotenoids, has also demonstrated some intrinsic antimicrobial activity against *E. coli* in the treatment of cotton fabric (Gonçalves et al. 2020).

Nanoparticulate antimicrobial agents, such as silver, CuO, and ZnO are frequently used for antimicrobial functionalities. Biopolymer-based nanocomposite finishes act to prolong or make the effects of nanoparticles more prominent. For example, (Xu et al. 2018) demonstrated that the covalent binding of carboxymethyl chitosan onto the cotton fabric surface enhances the adhesion ability of the Ag nanoparticles. Antibacterial activity was maintained after 50 wash cycles, and the treatment did not affect properties such as tensile strength, water absorptivity, and vapor permeability.

The ability of alginate to form resistant films by cross-linking with divalent ions has been explored to entrap and release active antimicrobial compounds. In general, the release and antimicrobial activity from treated fabrics show a negative dependence on the amount of alginate and the cross-linker concentration.

UV Protection and Multifunctionality

The UV protection functionality is one of the most important for textiles used under prolonged sun exposure, such as sportswear and beachwear. For this purpose, TiO₂ and ZnO nanoparticles are among the most explored compounds thanks to their intrinsic property of UV protection. Other inherent properties of these nanoparticles include photocatalytic activity, antimicrobial effect, and flame retardancy, which can provide multifunctionality to treated fabrics. The use of these nanoparticles combined with biopolymer coatings, especially subjected to cross-linking, aims to prolong the durability of the functionalization in the face of successive washings.

Agricultural Applications

The expansion of the world population and industrialization have been accompanied by increased plastic consumption for the most diverse uses and applications. The versatility, attractive price, and stability of plastic materials have contributed to disseminating their use in almost every sector, including agriculture. Plastic in agriculture includes greenhouse covers, mulches, low tunnel films, drip irrigation tape, plant containers, nets, silage bags, trays, seedling bags, and seed tapes (Adhikari et al. 2016; Gamage et al. 2022). In 2020, the sector consumed about

11.7 million tons of plastic worldwide, comprising about 3.3% of the total plastic production (Plastic Europe 2021). In the majority of cases, conventional fossil-based polymers are used, which, in the long term, can affect soil properties and non-biodegradable residues accumulation. Therefore, biodegradable polymers have increasingly been considered as substitutes for plastics in many agricultural applications, such as mulching, plant containers, and seed coating owing their natural degradation and soil-enhancing properties (Santos et al. 2020).

Mulching

Plastic mulching films have advantages for crop production, from both agronomic and phytosanitary points of view. They contribute to weed management, reducing the need for chemicals, favoring the crop by conserving soil moisture and moderating its temperature, and avoiding direct contact of fruits and leaves with dirt and soil. The most commonly used material for conventional mulching is low-density polyethylene, which provides an effective moisture barrier and radiometric properties, and mechanical resistance. As already mentioned, despite all the benefits, the fate of non-renewable and -biodegradable polyethylene mulches after their use is a major environmental concern due to a large amount of waste generated. The mulches must be removed from the field after use, involving dispendious hand labor or specific machinery. Farmers often discard the plastic residues in a dump or burn them, generating toxic emissions to air and soil (Immirzi et al. 2009).

As an alternative to contour those effects, biopolymers can be used to develop novel materials. The ideal plastic mulch would stay intact in the soil until the end of the crop cycle and then undergo full biodegradation within a few months. Currently, great interest has focused on the polysaccharides such as starch, cellulose, chitosan, glucomannan, and sodium alginate for mulching purposes. Cellulose has gained considerable interest because it is the most abundant natural polymer and is easily available. Chitosan and alginate, mainly derived from aqueous resources, exhibit excellent film-forming properties and biocompatibility. Agroindustry wastes and by-products can be rich sources of these biopolymers, contributing to the sustainability of the production chains and cost reduction.

Biopolymer mulches can be processed by extrusion, casting, and spraying methods to form biodegradable films and coatings from biopolymers (Adhikari et al. 2016). Compared with conventional options, higher costs and poorer mechanical and barrier properties remain the main obstacles to bio-based mulches dawning. However, more strict environmental regulations regarding plastic production and disposal, allied to the increasing demand for more sustainable production processes, are pushing researchers and organizations in the race for the development of competitive, environmentally benign materials (Santos et al. 2020).

Several strategies to improve biopolymers' properties have been proposed, such as chemical modification, cross-linking, blending different biopolymers, adding plasticizers, and loading with reinforcement filler. Cellulose fibers, wheat bran, dry algae, and other natural components were added to the polymeric solution to

enhance mulch properties (Adhikari et al. 2016). Sprayable biopolymer formulations for soil coating are also gaining special attention. For mulching purposes, spraying the biopolymer solution in the soil to form a protective layer instead of applying a pre-formed structure represents an innovative approach (Immirzi et al. 2009).

Bio-based films and coatings can also vehiculate active agents as nutrients, pigments, and growth promoters. A chitosan solution sprayed on the soil surface of plant pots efficiently inhibited the growth of weed species for about 3 months when it began to biodegrade (Giaccone et al. 2018). Alginate and glucomannan mulching films enriched with sugarcane vinasse were produced and characterized, allowing the recycling of vinasse nutrients into the soil. Those films were cross-linked with calcium ions reducing drastically their water solubility (Santos et al. 2020). Starch and chitosan blend films with the addition of citric acid and black pigments showed potential for application as biodegradable mulching for short-cycle vegetables and flower crops (Brandelero et al. 2019).

Containers and Bags

Plant pots, seed trays, and bags are widely used in horticulture and nursery for growing, packaging, and transporting purposes. Considerable large amounts of plastic waste are generated from those applications (mostly single-use), which are often left in landfill or burned due to difficulty and high costs for proper disposal and recycling. Bio-based polymers have gained importance in this segment as alternatives to petroleum-based materials with the advantages of significantly reducing the amount of waste, labor costs, and environmental impacts since they can be planted with the seedling or young plant and undergo biodegradation direct in the soil, eliminating the need for transplanting and disposal. Biopolymers derived from agroindustry by-products and wastes such as starch, cellulose, soy protein isolate, alginate, and pectin, associated with natural reinforcement fillers such as wood pulp, coconut husks and fiber, and rice hull, can represent innovative and sustainable options for the development of new products. Pots of alginate and fibers from tomato and hemp were produced for transplanting plants, and field experiments showed adequate root and plant development with complete degradation of the pot structure after 2 weeks (Schettini et al. 2013). Blick et al. (2014) developed starch/polybutylene adipate terephthalate (PBAT) biodegradable seedling bags and showed that the material remained intact throughout the seedling production period (60 days) but was completely biodegraded after transplantation into containers (240 days). Agonomically, there were no significant changes in plant dry mass compared to control (with no bag).

Seed Coating

Seed coating is a kind of seed treatment in which polymers are used as carriers of nutrients, antimicrobial, and other active ingredients, favoring seed performance in

terms of physical, physiological, and health quality (Rocha et al. 2019). Biodegradable polymeric matrices can significantly reduce the amounts of agrochemicals through their encapsulation in super-low volumes and controlled release, avoiding exposure of the product to the external environment, as well as its evaporation (Pirzada et al. 2020). The use of biopolymers as seed coating has been highlighted in scientific research, such as chitosan, which promoted increases in soybean seed germination and seedling productivity (Zeng et al. 2012), cellulose acetate (Friuli et al. 2020), and in addition to biopolymer mixtures that favored corn seed germination (Vercelheze et al. 2019).

Conclusion

In the last years, much effort has been made to replace synthetic polymers with biodegradable materials for ever-expanding applications. Unique properties, such as biocompatibility, biodegradability, nontoxicity, and the possibility of chemical modification, make biopolymers attractive for many fields, including food packaging, biomedical, textile, and agriculture.

The shelf life and quality of fruit and vegetables, meat, dairy, and seafood products can be improved with edible films and coatings. There is a major focus on developing packaging systems containing antioxidants and antimicrobial agents, enzymes, or functional ingredients. In medicine, surface modification of implants to meet required or add specific functions, such as cell proliferation, tissue growth, repair, and delivery growth factors, active molecules, antimicrobial agents, and drugs, can be achieved by biopolymeric coatings. Orally disintegrating films have emerged as a route of drug administration that does not need water to be ingested, which is particularly useful for pediatric and geriatric patient populations. Biopolymers play a significant role in this application since film must be composed of a nontoxic and water-dissolving polymer. Biopolymer films also represent a versatile platform for improving skin healing due to the ability to remove wound exudate while maintaining a moist environment and minimizing bacterial infections. In textile applications, biopolymer coatings, mainly nanocoatings, provide products with high added value, with flame retardant, antimicrobial, and ultraviolet protection functionalities. One of the biggest challenges for this application is the durability of the treatment, which tends to be removed with successive washes if there is no permanent bond between the coating and the textile substrate. For agricultural applications, biopolymer films and coatings act to reduce the amounts of synthetic plastics and agrochemicals, enhance soil properties, and load active agents.

In general, incorporating nanofillers and combining different biopolymers have been proved to be an economical and versatile way to yield films and coatings with improved properties, overcoming mechanical and stability limitations. However, more studies must be done in terms of scaling-up processes to increase the competitiveness of biopolymeric films and coatings.

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Gas Barrier Properties and Applications of Nanocellulose-Based Materials

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Nisha Nandakumar and Ajalesh B. Nair

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Abstract

Cellulose is a biomass that occurs abundantly in plants, animals, or bacteria. It can exhibit enhanced gas barrier performances due to their high degree of crystallinity and polar nature. Its linear polymer chain built out of β -D glucopyranosyl units linked via 1,4 positions and its fibrillar nature adds to its performance in slowing down diffusion of gases such as O_2 , CO_2 , N_2 , and vapor. During the last decade, this bio-nanomaterial was identified as a potential filler in composites to taper its reinforcement capacity. “Nanocellulose” refers to the cellulosic materials whose structure falls to the nanoscale regime. Its dimensions falling in nanoscale and formation of strong entangled nanofibrillar network and porosity, have inspired the evolution of novel applications. They may be cellulose nanocrystals, fibers, or bacterial cellulose. Nanocellulose is a non-hazardous, biocompatible, degradable

N. Nandakumar · A. B. Nair (✉)

Department of Chemistry, Union Christian College, Aluva, India

Department of Chemistry, Sri Vyasa N. S. S. College, Wadakkanchery, India

e-mail: ajaleshbair@uccollege.edu.in

mass without side effects on health and environment. In previous years, as a result of advancement in research-based optimizations, its mode of synthesis has transformed a lot. New biosources, new mechanical processes, and new strategies and treatments have opened up a new realm in nanotechnology. Pharmaceutical packaging shows deadly demand for biocompatible oxygen barriers for retaining moisture and freshness, so do the need exist with electronic industry. Here we are focusing on nanocellulose-based gas barriers fabricated by itself via a suitable method such as layer-by-layer formation; nanocomposite was fabricated using extrusion, electrospinning, casting, or evaporation, where its primary role is reinforcement in those polymer matrices or as hybrid composite with prominent nanomaterials. The challenges in this realm are the water absorptivity and swelling or geometrical deformation of nanocellulosic films or composites and henceforth the failures in meeting material applications. In this chapter, nanocellulose-based gas barriers and its applications in various technological and industrial realms are discussed.

Keywords

Nanocomposites · Cellulose · Membranes · Gas barriers · Hybrid composites

Introduction

Recent progression in science and technology coupled with an increased nature consciousness has moved the societal and industrial foci toward green mass and eco sustainable process. This agitation has been fueled by the depleted oil mines and the imbalance existing between the production of polymers and its recycling. As a driving force there is an option to replace oil-derived polymeric materials with renewable counterparts, preferably a biodegradable material. However, a complete replacement is not an easy task because the good material properties and excellent processability offered by these polymeric materials stabilize their market position. Common packaging materials and oil-based polymers possess good barrier properties, stability readiness to be processed, and high degree of transparency, which are just a glance of their desired qualities (Vermeiren et al. 1999). To catch oil-based polymers with renewable and bio-based materials, it is essential to reach at least similar application levels at a comparative cost. One mode to readily achieve this aim is to use naturally abundant biopolymers with existing industrial-scale production and processing protocols.

Moreover, huge amount of plastic packaging wastes produced yearly aggravate the pollution of marine and terrestrial ecosystem (Robertson 2013). Microplastics pose the menace of getting delivered to other organisms through food chain once it is ingested (Andrady 2011) because no organism can ever break it down or digest it. Along with this, materials of this kind induce excessive emission of gases that cause green house effect and henceforth lead to global warming. Global

environmental menaces due to petroleum-based plastics have become a serious concern. Apart from recycling used polymer packages, the fabrication of materials with biodegradability is the demand of the hour. On an environmental outlook, biodegradability is unavoidable apart from a functional requirement (Siracusa et al. 2008), especially considering that the bioplastics industry is growing at a rate of 20% per year (Peelman et al. 2013).

Gas Barrier Properties – Present Scenario and Green Barriers

By virtue of their barrier performance, thermoplastics, such as ethyl vinyl alcohol copolymers, polyvinylidene chloride, and aromatic polyamide, are used for extending food shelf-life by acting as barriers to small gaseous molecules. Compared to these, PVDC acts as better barrier to O_2 and water vapor in humid and freezing conditions. However, as shown in Fig. 1 (Nelson et al. 2016), the nanocellulose trends the highest oxygen barrier properties.

Copolymer of ethyl vinyl alcohol has more resemblance to nanocellulose (Fig. 1). The problem associated with both these barriers is that their resistance to passage of gases adversely diminished when freezing or high relative humidity (RH) is generated. Due to the plasticization mechanism, free volume increases owing to the swelling of $-OH$ groups present in their respective structures (Nelson et al. 2016). Copolymer of ethyl vinyl alcohol is a thermoplastic substrate which can be laminated in multi-layered hierarchical structures, whereas a neat nanocellulose is not

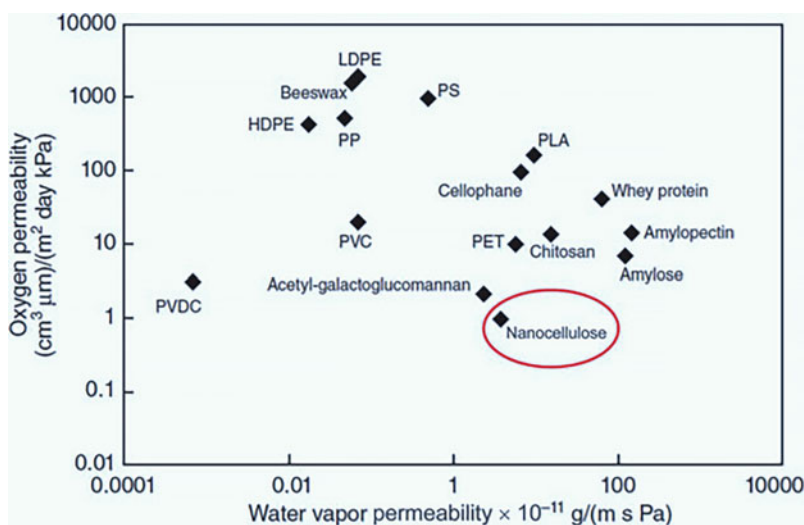
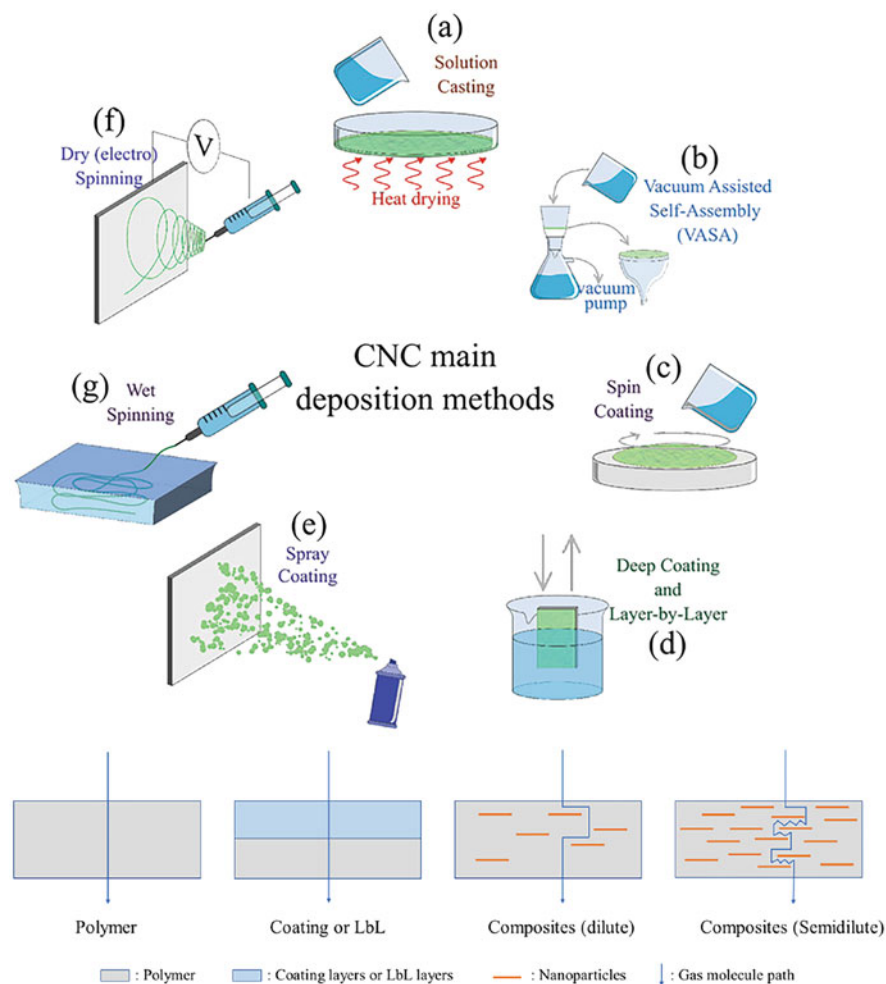


Fig. 1 Barrier properties of nanocellulose, synthetic plastics as well as bioplastics, and other bio-structures – their KPO_2 , KPW_2O (unit – $cm^3 \mu m/Pa \text{ day } m^2$ and $cm^3/day \text{ m } Pa$), respectively (from Nelson et al. 2016), oxygen and water vapor resistance coefficients focused

thermoplastic, so, need a blending with any combination of thermoplastics to fit their application as a packaging material. Lamination technique helps to alleviate the hydrophilicity of copolymer of ethyl vinyl alcohol and makes them fit to use in flexible materials. Nanocellulose improves the water vapor permeability (WP) of thermoplastics (Rhim et al. 2013) and cross-linked composites and restricts the swelling of hydrophilic polymer or nanocellulose at high humidity. It is known that water absorption weakens the high gas barrier as well as other beneficial properties for both nanocellulose films and composites. This poses the biggest menace as far nanocellulose-based materials are concerned. They possess perfect degree of orientation and mechanical strength but removal of water at pH ranging at four takes more than 4 h to making scaling-up a tough task. However, these findings are precious as they show the predominance of nanoscale structures and the trade-off between nanodimensions and processing time when focusing on overall properties. Aiming at enlarging the spectrum of biomaterials, several researchers have been focusing on novel bioplastics, compounds, and batches. A number of these polymers have been made available in a commercial scale for food packaging purposes such as aliphatic polyesters, poly(lactic acid) (PLA), starch, and cellulose (Siracusa et al. 2008), especially, chitin and chitosan. Polyesters are very crucial, as most are biodegradable and biomass based. Nanocellulose is a bio-based nano-polymer which is readily degradable, renewable, and eco-friendly material. It is made from a number of sources of natural origin such as plants, animals, and bacteria (Chen et al. 2011; Du et al. 2021). Usually reported forms of nanocellulose are of two: nanocrystals (CNCs) and nanofibrils (CNFs). CNCs (Foster et al. 2018; Moon et al. 2011) are rod-like particles, while CNFs are fiber-shaped ones (Chen et al. 2019). Other nanocelluloses are microcrystalline cellulose (MCC), microfibril cellulose (MFC) (Ewulonu et al. 2019), bacterial nanocellulose (BNC), and cellulose nanosphere (CNS). All these nanocelluloses consist of crystalline and amorphous regions (intermittently arranged). Usually, CNC has 72–80% crystallinity which is higher than that with 50–65% crystalline CNF, convincing the point that higher rigidity of materials and superior gas barrier potential is possible with these (Ferrer et al. 2017; Wu et al. 2021). Nanocellulose has strong hydrogen bonding interactions which influence physical–chemical properties and is hence hydrophilic. Nanocellulose, single CNF or CNC, shows strong mechanical strength and shows increased modulus and tensile strength. All these properties enhanced over the longitudinal direction of samples under study. Transparency is a property on demand in packaging applications due to requirement of content visibility. Nanocellulose films show high mechanical properties than the general spectra of polymers or synthetic polymers such as PET, LDPE (Aulin et al. 2012), and PS. The study of properties of filled polymer composites shows that incorporating nanocellulose into the polymer (generally called matrix) enhances their mechanical strength. Nanocellulose exhibits good optical performance. Optical transmittance value tops up to 90% (Aulin et al. 2010) and even close to 100%. Nanocellulose in the matrices does not deplete the optical texture of these materials (Espino-Perez et al. 2013). Mechanism of gas diffusion may be schematically shown as in Fig. 2.



Ash block—Polymer, Blue layer—Nanocoating or LbL, Blue line—Path of Gas molecule, Red line—Nanoparticles

Fig. 2 Cartoon of the most common nanocellulose-based composite preparation methods (a–f) above and probable scheme of gas diffusion through polymer and composites below (Wu et al. 2021)

Gas Barrier Performances of Different Nanocellulosic Materials

Cellulose Nanocrystals (CNC)

CNCs are rod-like dwarf materials with low degree of entanglement, particularly for CNC films with good degree of order, which leads to brittle (Chowdhury et al. 2019) films and are prone to large crack formation. The CNC film surface is much

smoother than that of CNFs. CNC-reinforced composites are more efficient as property enhancers in gas barrier performance due to their higher crystallinity. Films of CNC and CNF are good gas barriers as well. Water vapor permeability (WP) of films of nanocellulose is appreciably high due to good water uptake characteristics (Ferrer et al. 2017). The oxygen permeability of CNC films studied at different crystal orientations revealed that rod coater provided CNC films with unique order (Chowdhury et al. 2019). Ordered films are seen to exhibit a value of $0.2500 \mu\text{m}^2 \text{ day kPa}$, which is lower than the value shown by randomly arranged CNC films which comes as $219.00 \mu\text{m}^2 \text{ day kPa}$. Anisotropy affects gas permeability appreciably. In quest for creating gas barriers and active material for packing, a technique named LBL (layer-by-layer processing) has been practiced. In LBL, they interacted nanocellulose with some cationic polyelectrolytes. They may be selected from common cationic polyelectrolytes like chitosan and polyethyleneimine (Foster et al. 2018; Miao and Hamad 2013) and polydiallyldimethylammonium chloride or polyamidoamine resin. Combination of multilayers shows highly versatile coatings of ready-to-use structures such as boxes, plates, and bottles. The gas permeability of CNC films is superior to CNF or MFC films (Belbekhouche et al. 2011) and the CO_2 , N_2 gas permeability of CNC films is 1000 times higher than that of films of CNF, which owes to the lower entanglement between CNC and film brittleness. By adding minor amounts of CNF into these kinds of CNC films or increasing the degree of order of rod-shaped CNCs, permeability of CNC films decreases. It is due to the change in the packing density as well as the free volume of the system containing CNCs (Chowdhury et al. 2019; Fotie et al. 2018).

Cellulose Nanofibrils (CNF)

Numerous studies tried to solve water absorption-related problems, and few of them showed good results, but a few argue that not all methods could alter and affect the barrier property. The hydrophobic modification of quaternary alkyl ammonium salts in a study indicated that OP is directly proportional to molar ratio or chain length of quaternary alkyl ammonium salts. Modification is a solution that can certainly add hydrophobicity to films and delay water uptake, but it compromises the film density which directly induces an increased free volume of these CNF films, which initiate and propagate ready transport of the O_2 molecules and hence an increased OP. CNF films being developed as entanglement of nanofibers have good mechanical and gas barrier properties. This superior behavior owes its origin from high aspect ratio of these celluloses (Visanko et al. 2015; Herrera et al. 2014). Water vapor permeability of CNF films (Saengerlaub et al. 2019) is about 1000-fold higher than that of polymeric ethylene and ethylene glycol (Li et al. 2019). Hydrophobization by modification of nanocellulose can decrease the WP. An example for this is 10-undecylenoyl chloride (Li et al. 2019), which has superior water vapor resistance. However, hydrophobic modification has limitations in certain cases. It reduced the interaction between CNF chains (Solala et al. 2018), ending up in higher free volume of the films, which subsequently worsens water vapor barrier properties. Thermal

treatment reduces porous structure, and increase in crystallinity improves water vapor barrier properties, which is already a proven OP barrier. Other than oxygen, barrier to other gases is also significant. Carbon dioxide permeability of nanocellulose film is lower than that of low-density PE (Chowdhury et al. 2019). PP and PVC are in the same range of copolymer EVA. The CH_4 (Belbekhouche et al. 2011), CO_2 (Ansaloni et al. 2017), and N_2 gas permeate in a rate which is proportional to increase of RH.

Bacterial Nanocellulose

Bacterial cellulose (BC) is a useful cellulose nanostructure used either as reinforcing phase or as matrices for a wide range of variety of materials including films for food packaging applications. *Gluconacetobacter* synthesizes nanocellulose in a medium with C and N sources. Chemically, it is identical to plant cellulose; the bacteria synthesize cellulose and form group of nanofibrils and assembly of nanosized ribbon-shaped fibrils with <80 nm in width. It is also free from hemicellulose and lignin, reducing purifying costs and environmental damages of those derived from using harsh chemicals. It can form a pellicle membrane-like structure with a water retention capacity of nearly 700 times its dry weight. These membranes may be impregnated with polymer dispersions having reinforcing agents and antimicrobials. A bottom-up technique was used to self-assemble nanocomposites in which BC was grown in the presence of a biopolymer mixed in the medium. Works reported that these BC nanowhiskers (BCNW) filled in polyhydroxybutyrate-co-hydroxyvalerate (PHBV) matrices reinforced and had highly dispersed structure with better reduction in oxygen permeability without much change in mechanical performances.

Microfibrillar Cellulose (MFC)

From the pulp of bleached spruce sulfite, MFCs were synthesized and their oxygen permeability data were consolidated. They prepared MFC films without specific orientation using a 0.10% MFC suspension in a cylindrical mold, dried at room conditions. They were 20–33 mm thick, and their corresponding weight was found to be between 15 and 30 g/m². The OP values measured at 0% relative humidity were 17–18 mL/m² day. OP of the copolymer or PVDC-coated or -oriented polyester ones falls in the range of 3–5 mL/m² day and 9–15 mL/m² day. These films are therefore superior to several well-known synthetic polymer films due to their dense and nanostructure with porous network. After these observations, carboxymethylation pretreatment on MFC fibrils evidenced that they were highly charged and ready to liberate. This was evidenced at an RH of 50%; here the pretreated MFC films presented OP value of 0.085 mL mm/m² day atm. This is superior to the values for neat MFC which is between 0.3520 and 0.5050 mL mm/m² day atm (Aulin et al. 2010). This work also highlighted the impact of different parameters connected with synthetic strategy applied during MFC production. The films unlike the raw

low-charged MFCs had a tightly packed network formed by fibers with smaller and more homogeneous structural parameters. It was noticed that as the RH raised from 0% to 50%, a considerable increase in the OP values could be observed, from 6×10^{-5} to 0.0850 mL/mm m² day atm, respectively. The crystalline structure of MFC and its ability to form tightly packed dense interfibrillar network as well as intrafibrillar hydrogen bonds adds a lot to its ability to resist gas diffusion. At a low relative humidity, such an OP can be attributed to these structural features. At a higher RH of about 70%, the hydrogen bonds loosen, creating films that are less densely packed. Even at such a high RH, however, they provide better OP values that are superior to those of other biopolymers and films. Other parameters studied were the weight and the thickness of films which indicated that as the weight and thickness increased, the difficulty of the path that oxygen must traverse also increased which resulted in a decrease in the OP value. Moreover, the degree of OP also decreased with increased film thickness. Obviously, most pores are located at the surface of MFC films, the pores are scattered and thus contribute to the impermeability of films when they have proper thickness. The OP value of neat MFC films from kraft pulp was about 4 mL/m² day corresponding to a weight of 30–40 g m⁻². Higher OP values depending on the reaction time (from 6 to 11 mL/m² day) were seen for acetylated MFC films. Regardless of the treatment, whether it be acetylation or carboxymethylation or another one, under a given experimental condition to produce MFC films, they are seen forward as new biomaterials to realize the concept of good barrier food packaging. Compared to other renewable material including different cellulose and synthetic polymers, MFC films possess low barrier properties. The degree of crystallinity is high for MFCs that they even exhibit a OP that is much superior to that of cellophane. Fukuzumi et al. (2011) cast a TOCN/water dispersion to PLA film. It was a plasma-treated one and this methodology was found to modify MFC's affinity with water preserving its good gas permeability. The obtained TOCN-coated layer had 0.4 mm thickness on a 2.5-mm-thick PLA film. These ultra-thin MFC layers had a significant decrease in the oxygen permeability (from 7.4 to <0.001 mL/m² day kPa) between an unmodified PLA film and a PLA film. Similar conclusions have recently been reported, based upon the coating of PET films with TOCN dispersion, which decreased the OP value from 29.3 to 0.08 mL/m² day (Rodionova et al. 2011). The same year, a similar coating process for PET films and two kinds of TEMPO-mediated oxidation were proposed by Fujisawa et al. They prepared the usual TEMPO/NaBr/NaClO-treated cellulose nanofibrils (called TOCNs) with sodium carboxylate groups (COONa), and TEMPO-oxidized cellulose nanofibrils, HCl washed with free carboxyl groups (TOCN-COOH). The PET films were then coated with a thin layer of TOCN film in order to access their oxygen barrier properties. The oxygen permeability of these films decreased from 0.31 to 0.049 mL/m² day kPa when coated with modified form. Regarding the oxygen permeability, the combination of MFC with a polylactic film (Fukuzumi et al. 2011) and an amylopectin solution (Plackett et al. 2010) reported a decrease in this property. In this regard, two different MFCs with high amount of hemicelluloses and other with carboxymethylated MFCs (Plackett et al. 2010) were prepared, and amylopectin was added. Depending on the ratio of MFC to

amylopectin and also the type of MFC, the average OP (at 23 °C, 50% relative humidity) varied between 0.013 and 0.037 mL mm/m² day atm. The results obtained for amylopectin films with a 50 wt.% as well as 100 wt.% MFC films were similar. All the composites displayed reduced permeability values in the range 0.01–0.02 mL mm/m² day atm. These values are low on comparison with the oxygen permeability of amylopectin film which is 1.40 mL mm/m² day atm. Consequently, the addition improved this property, and the case repeats with the other combinations with MFC. So these combinations can lead to nanocomposite with good oxygen barrier. A nanopaper was prepared by coupling layered clay with MFC, which produced a strong and good gas barrier. When relative humidity was as high as 95%, a drastic increase of OP to 3.5 mL mm/m² day atm could be noted, whereas at 0% relative humidity and 50% relative humidity, low values were confirmed when half MFC and half clay combinations were studied (0.001 and 0.045 mL mm/m² day atm, respectively) and for 100% neat MFC films, the value showed an exponential increase from 0.048 to 17.5 mL mm/m² day atm.

Nanocellulose Hybrid Materials

A number of hybrid nanocellulose materials are explored which exhibit superior barrier properties. Excellent gas barrier film was fabricated by LBL using combination of polymer with nanoparticles or polymers having layer thickness in the range of hundreds of nanometers (Ariga et al. 2007; Hammond 2004). Nanoplatelet materials are promising barriers and hence are widely used as gas barrier layers due to the high surface area these platelets possess (Choudalakis and Gotsis 2009). The LBL technique is seen to improve gas barrier properties of these nanocellulosic materials. This is due to the concept that nanocellulose makes it feasible to be deposited as a very thin layer in a preferably parallel direction to the surface of the prospect film, leading to nanocellulose assembled in a perpendicular direction to the path of gas permeation and hence forms a barrier system. Materials such as (GO) graphene oxide, gibbsite (Chemin et al. 2019), Guar gum (Dai et al. 2017), alginate, clay, chitosan, and polyelectrolyte were prepared similarly with CNF (Li et al. 2013) or CNC to create gas barrier films (Halasz et al. 2015). By increasing the number of deposited layers, gas barrier properties of LBL materials can be tailored and enhanced barrier properties are observed after several bilayer depositions are executed. OP of gibbsite/CNC LBL films reaches 75% reduction by seven bilayers as in Fig. 3, and chitosan/CNC LBL films (Li et al. 2013) show 94.0% reduction at the 30 bilayer deposition (Chemin et al. 2019). It is reported as the oxygen permeability of polyethyleneimine/nanofiber film marks 94.0% deduction, whereas water vapor resistance reaches 52.0%. There is a 0% deduction by 50 bilayers when compared to the uncoated substrate (Aulin et al. 2013). This is mainly due to swollen structure, and hence, an improvement of WP ability is more significant as compared to the OP. In addendum to the increased number of bilayers, low air voids are used to achieve homogenous nano-coatings, which helps to improve barrier properties. For a given polymer cellulosic nanoassembly, there is an increase in film packing density

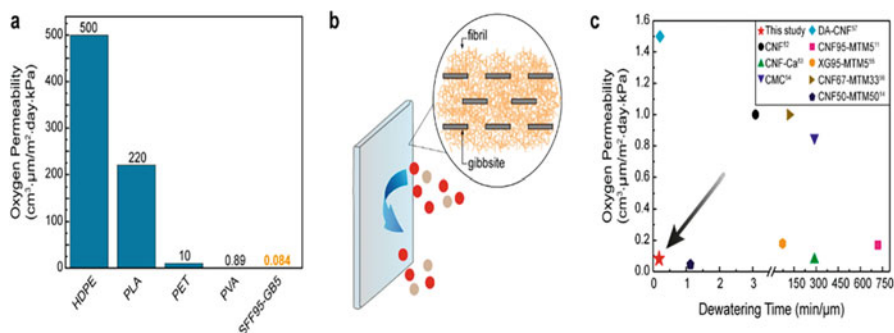


Fig. 3 (a) OP – SFF95–GB5 versus conventional plastics used in packaging (*HDPE* high-density polyethylene, *PLA* polylactic acid, *PET* polyethylene terephthalate, *PVA* polyvinyl alcohol). (b) Pathway for enhanced barrier properties. (c) Graph for oxygen permeability and dewatering time normalized by thickness for control and for wood-based materials. From literature, dramatic improvement in the OP of cellulose nanocomposite films is achieved with GO loading, as seen from Table 1

Table 1 Regenerated cellulose (RC)–graphene oxide (GO) films and their permeability to oxygen

Serial no.	Designation of composite films	PO ₂
1.	RC	$1.59 \times 10^{-12} \text{ cm}^3 \text{ cm/cm}^2 \text{ s Pa}$
2.	RC/1GO	$4.63 \times 10^{-14} \text{ cm}^3 \text{ cm/cm}^2 \text{ s Pa}$
3.	RC/5GO	$1.8 \times 10^{-15} \text{ cm}^3 \text{ cm/cm}^2 \text{ s Pa}$

by nanocellulose and polymer interactions between layers. The gas barrier property improves because a barrier effect is imposed on the polymer in the presence of partially impermeable semi-crystalline structure of nanocellulose. The LBL assembly alters OP much better than that of water vapor barrier properties as reported in earlier works (Dai et al. 2017). The nanoplatelet- and nanocellulose-filled polymers fabricated using LBL strategy show an excellent improvement of barrier properties. A previous work studied that LBL casts graphene oxide-CNF films – having 3.66 vol.% GO, which results in the OP coefficient of CNF film to decrease by about 40,000 times. Qin et al. reported that permeability of clay-filled CNF had permeability orders of magnitude lower than the neat substrate when 20 bilayers were deposited. Nanoplatelets in the meanwhile can also block water molecules when filled within such polymers that are capable of decreasing water absorption of the nanocellulose, resulting in high gas barrier properties even at high relative humidity conditions of operation. In a reported work, blending CNF/alginate to form films shows huge CNF aggregation and hence possesses poorer air barrier properties than bilayer or multilayer films fabricated with alginate itself. The same phenomenon could be observed with few other systems as can be seen in CNF/clay systems. In these, the blended composites of CNF/clay exhibit superior permeability values than the layered matrix of the same materials. Layered assembly can achieve nanoparticle arrangements that are well orientated in the polymer systems and thus

prevents aggregate formation of these nanoparticles and hence results in superior properties compared to the blended-type composites, as far as barrier capacities are concerned. However, these blending materials are readily processible and suitable for a wide range of polymers and nanoparticles than the former LBL materials. Nanocellulose consists of both polymeric part and nanoparticle part, which can play dual roles depending on the nature of different systems into which it is incorporated. Incorporation of different shaped nano's or varied components into the NC (nanocellulose) system yields various NC composites with different mechanical, optical, morphological, and barrier properties. Different particles such as clay, silica, layered double hydroxide–nanocellulose composites were studied for gas barrier properties. Nanocellulose composites with boron nitride nanosheets, halloysite structures, MWCNT, SWCNTs, nanographene, etc. were also studied. Silicates especially clay being a 1D nanoparticle of inorganic origin have a diameter of 30 nm to microns and about 1 nm thickness (Hu et al. 2020). The clay/nanocellulose films processing is done by simple mixing of these two raw materials in a suitable solvent, fixing it and filtered suitably and finally dried to obtain the final material. The layered structure of hybrid clay/nanocellulose composites is highly ordered and resembles platelet pack and have the nanocellulose matrix cover around it. The schematic nanostructure and SEM of the clay/NC composites are as shown in Fig. 3. The materials that resemble or imitate natural phenomena are drawing great interest since these hierarchical structures can yield superior, mechanical properties, stiffness and toughness, flame, heat retardant, and gas barrier abilities. Looking into the clay–NC composite films, there is markedly a continuous decrease in OP with clay content up to 50 wt.%. A reduction in OP in a range of about fourfold times could be seen with 1 wt.% clay, and this marks about 40-fold reduction when 50 wt.% clay was incorporated, all achieved at 0% relative humidity. Thus it's interesting to note that both barrier properties enhanced in a hand to hand mode with increasing the clay content in nanocellulose composites. In the case of CNF films, pure films show super high permeability when at a high relative humidity of 95%. This may be because at certain water levels, there will be disruption of hydrogen bonding and van der Waals interactions. On the other hand, as seen earlier the clay in clay/NC films helps to build barrier layers to water molecules and helps in reduction of the water absorption by nanocellulose. Though this is not an ultimate solution to water absorption-related issues, the composites still showed higher permeability at high humidity conditions compared to the dry conditions which is a ray of hope to futuristic prospects in application of these hybrids films. Crosslinking of or addition of positively charged additives produces more compact materials to generate even higher barrier materials. As seen with chitosan act as a flocculant in co-dispersion of negatively charged NFC owing to its positive charge and clay and/or surface modification with cationic groups as seen with CNF helps to self-assemble it with anionic clay forming composites with more densely packed structure. Gas permeability studies of reinforced composites (Fredrickson and Bicerano 1999) were published for all impermeable materials as plate-like, spherical, and tube-like nanoparticles. These helped to improve the microstructure of the clay/nanocellulose composites and exhibit the direction for futuristic research and technological aspects.

The barrier layer was composed of sheets, which significantly reduces the usable area parallel to the film and the compact structure promotes the formation of the large gas barrier layer of GO. Further it expands the gas diffusion path. As seen from Table 1, GO effectively suppress the penetration of O₂ into the composite films. Significantly, the OP of the RC (regenerated cellulose) film is as low and is almost 34 times higher than that of the RC/1GO film. As the GO content increases, a further steady decrease in OP is observed with increasing GO loading from 1% to 5%. The RC/5GO film shows about considerably 388 times reduction relative to the regenerated cellulose film and RC/1GO. Hence, the gas barrier performance of the NC film significantly improves due to the strong interfacial binding force between GO/NC (nanocellulose) matrix.

Nanocellulose-Reinforced Composites

Extrusion of nanocomposite is a general process where a continuous profile of the matrix is made into desired thickness by incorporating fillers into it by melting raw materials at a given temperature. The matrix made into desired thickness gives a modified polymer or composite of fixed shape on cooling once they are forced into a die. Recently, as a filler for the mechanical reinforcement of PLA adhesives and polymers, nanocellulose plays an important role. These matrices take the advantage of the very high specific surface area and aspect ratio of these nanocellulosics to boost their performances. The mechanical properties of low-density polyethylene when extruded with esterified CNCs grafted with acid chlorides of organic compounds found to improve, especially the elongation at break showed a marked improvement. Casting is a general operation of removal of solvent via evaporation from the NC at a given temperature to carry out evaporation in a controlled manner. Through this process, dried films of neat or modified NC are obtained. It is important to note that CNCs are brittle compared to CNFs which are flexible; but suitable plasticizers if incorporated can compromise the brittleness by improving the capacity of the CNC structures by reducing formation of hydrogen bonds which is seen reported for addition of sorbitol and glycerol. A composite melt is also made out of polymer by heating, either resistance heating or circulating fluids can be done. Fibers up to macrometric scale is prepared by electrospinning, which is a technique that uses the electrostatic forces of the fiber. In the past years, studies conducted showed that the pattern of orientation of the fibers that occurs during the ES alters the thermo-mechanical properties and crystallinity of the ES fibers; this very same concept has been successfully employed for exploring cellulose-based materials. CNC and MFCs were incorporated into PE and PVA via electrospinning method, and the respective electrospun complex matrices showed higher compatibility and improved mechanical properties. Very recently, similarly processed composite films of nanocellulose and poly (hydroxyalkanoates) showed good water impermeability. Clay-filled composites still show superior performance among different class of available fillers. Nanocellulose/montmorillonite composite films prepared from radical form of 2,2,6,6-tetramethyl substituted piperidine-1-oxyl (TEMPO)-oxidized

nanofibrils of cellulose (TOCN) with these filler nanoplatelets. The composite films obtained possessed good transparency and flexibility exhibiting superior mechanical strength and oxygen permeability resistances. By casting of a mixture formed of cellulose nanocrystals and plasticized starch, a nanocomposite material has been successfully obtained. The cast structure resulted had a remarkable enhancement of water resistance and mechanical properties such as tensile strength and Young's modulus from 3.9 to 11.9 MPa and from 31.9 to 498.2 MPa, respectively (Cao et al. 2009). Casting is not commonly used in packaging, but following the casting, CNC films were found to be transparent and denser.

Summarized Comparison of Barrier Performances of Nanocellulose

Frequent studies conducted as seen in literature regarding nanocellulose composites and majority of these concentrates on mechanical properties and extraneous applications. Only a few reports are found to study the barrier to gases and water vapor as well. Nanocellulose is directly added into the matrix of polymers, and the gas barrier properties of these nanocellulose composites are studied, the ultimate aim of which is to report a reduced OP of the polymer matrix. Polymer matrixes chosen are basically belonging to the group of thermoplastics and thermosetting plastics. Once filled in the appropriate matrix, the filler creates tortosity in gas permeation path creating harder diffusion roads for gas molecules and alters packing density inducing changes in crystallinity. These fillers also rearrange the water molecules in the matrix of the nanocellulose and hinder the plasticizing effect when present in polymers and thus increase matrix stiffness. The nanocellulosics generally employed to reinforce the polymer matrix are CNC and CMC having rod shape, fibrillar CNF and MFCs, and spherical CNSs. Thermoplastic matrices have been widely chosen as apt matrices to study the gas barrier properties of cellulose-filled systems. Nanocellulose-reinforced green biodegradable thermoplastics have become thrust area research subject, since these materials are best options as packaging and barrier materials for technical applications. Gas barrier properties of traditional thermoplastics reinforced with nanocellulose such as polymethylmethacrylate (PMMA), polyethersulfone (PES), polyvinyl alcohol (PVA), LDPE, polycaprolactone (PCL), polyester, and other class of thermoplastics such as sodium alginate, poly(butylene succinate), whey protein isolate, glutenin, gluten, polylactic acid, gelatin, thermoplastic starch, chitosan, agar, Guar, and collagen, all of biodegradable characteristics were analyzed. An increasing nanocellulose content in these filler-reinforced composites improves gas barrier properties on comparison with pristine polymers and the permeability decreased inversely proportional to the cellulose content. Gas permeability of PLA/CNC nanocomposites showed 31% and 67% reduction of vapor and oxygen permeability at a CNC loading of 3.0 wt.%. CNC and CNF particles are good in upgrading gas barrier properties of the polymers, even with low loadings of nanocellulose in these systems. To summarize recent papers, around 10–80% reduction of maximum permeability is observed with 1–20 wt.% nanocellulose.

Permeability shows the lowest point with a certain loading of nanocellulose and after that further loading of nanocellulose does not alter the resistance properties (Carvalho et al. 2018) in a noticeable manner. Guar/NC composites show decreased OP and increased WP (Dai et al. 2016) on increased addition of nanocellulose. OP and WP showing different trends may be correlated to water absorption or different RH values of the OP, that is, 40% RH and WP at 75% RH. It is possible that, at higher RH testing conditions, OP can also increase with the increasing nanocellulose content. Detailed studies are needed to explore the actual mechanism of this phenomenon. Other than the OP and WP, the permeability of other gases as CH₄, D₂, air, CO₂, and N₂ was also tested. The barrier properties of nanocellulose composites for these gases showed the similar trends as seen with OP and WP. Apart from all these fillers, modification of fillers was also found to be effective in affecting permeation. It was found that the silylation of CNCs in silicone rubber matrix works better than unmodified CNCs in reducing the polymer WP due to the improved affinity to the matrix. Silylation enabled crosslinking of otherwise less compatible CNCs into the matrix. However, some of the papers reported no gas barrier property changes or even increase of permeability of the thermoset composites filled with nanocellulose. The mechanism of gas barrier activity of nanocellulose-reinforced thermoset and that for thermoplastic are more or less the same, in which the nanocellulose creates tortuous paths for gas molecules passing through. If the nanoparticles are evenly dispersed to a good extent, it helps a lot in improving properties of thermoset materials. A poorly dispersed nanoparticle system on other hand may negatively affect the barrier properties. Henceforth, the gas permeability of these composites can be further improved by better dispersion of nanocellulose and improved inter-phase interaction between the nanocellulose and matrix.

Conclusions and Prospects

Nanocellulose is a bio-based polymer and hence a potential replacement to end plastic pollution due to packaging. To obtain super performing gas barrier materials from pure nanocellulosic materials, focus should be made on synthetic strategies and filler modifications. In combination with polymers as well as modified/unmodified fillers, even small amount of nanocellulose enhances gas barrier property and equips them to be more competitive with traditional pollutant packaging polymers. It can be used in pristine form or in combination with inorganic particles/polymers of bio origin. This chapter attempts to summarize recent studies on barrier properties of nanocellulose films, especially gas barriers, hybrid materials, and different composites. It concludes that nanocellulose-based matrices, films, and composites are useful for applications as packaging matrices or membranes based on their vapor, oxygen, and different gas permeation properties. However, application of nanocellulose materials is still challenged to overcome the hurdles as permeability, swelling in humid conditions, and improper dispersion of the nanocellulose in the polymer matrix. Some of the research papers recently published showed results concerning the rectification of these backdrops by modification or surface treatment of

nanocellulose. However, further studies and systematic strategies are needed to extensively use these materials in the industrial realms. The basic requirements of a matrix for packaging and technological applications are good tensile strength and better thermal properties. Apart from this, food packaging materials should meet some specific requirements, the ultimate function of which is to extend food stability, shelf-life, and to assure its quality/safety during that prescribed period. Barrier properties are so crucial as it focus on reducing gases and water vapor exchanges between the stored stuff and the environment outside it, decreasing the effect of physico-chemical factors and microbiological changes. Water vapor permeability (WP) and O₂ permeability (OP) have to be focused in depth and tailored for better performances. For a hydrophilic material, a decreased water solubility is of great importance, since a certain degree of water resistance is desirable for food applications to avoid film disintegration when in contact with humid food surfaces such as juices, meats, dairy foods, and fruit cuttings. Transparency is also usually desirable, so the consumer is satisfied with esthetics of the product. The objective of this chapter is to summarize observations and applications of cellulose nanostructures for packaging that are bio-based which can be extended to storing and coating purposes. A focus on the material properties related to packaging and barriers can be extended to superior levels using these advanced materials.

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Applications of Biopolymer-Based Nanofibers and Nanomembranes

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Arooj Ramzan, Azeem Intisar, Nazish Mumtaz, Nazim Hussain, and Muhammad Bilal

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Abstract

Biopolymers are referred to as the materials which are synthesized or produced from natural sources, such as plants, animals, microbes, or any other living entity. The use of these polymers has increased considerably in recent years as the industry moves away from unsustainable fossil fuel supplies and comes toward a softer and more sustainable eco-friendly approach. The significant information about biopolymer-based nanostructures is included in this chapter. Electrospinning is a new fabrication technique that uses strong electric fields to make biopolymer-based nanofibers and nanomembranes. This approach enables broad tuning of material properties and functions by adjusting the solution composition. A summary of the significant biological uses of these biopolymer nanostructures

A. Ramzan · A. Intisar (✉) · N. Mumtaz
School of Chemistry, University of the Punjab, Lahore, Pakistan
e-mail: azeemintisar.chem@pu.edu.pk

N. Hussain
Center for Applied molecular biology (CAMB), University of the Punjab, Lahore, Pakistan

M. Bilal (✉)
Institute of Chemical Technology and Engineering, Faculty of Chemical Technology, Poznan
University of Technology, Poznan, Poland
e-mail: muhammad.bilal@put.poznan.pl

is also presented, including medical implants, tissue engineering, wound dressings, drug delivery, and the transport of bioactive compounds, as well as additional environmental applications such as biosensors, filter membranes, wastewater treatment, and so on.

Keywords

Biopolymers · Nanofibers · Nanomembranes · Drug delivery · Tissue engineering · Wastewater treatment

Introduction

Nanotechnology has become a new multidisciplinary field of study in scientific inquiry. Nanotechnology has transformed the fields of technology and science in the twenty-first century, with far-reaching implications for human life and the economy of the future (Barhoum et al. 2019). Nanotechnology is the study of materials with sizes ranging from 1 to 100 nm. Nanomaterials are nanotechnology advancements such as nanofibers, nanomembranes, nanowires, nanotubes, nanorods, nanocrystals, and so on (Almetwally et al. 2017). These diverse nanoparticles have potential distinguishing features. Nanomaterials have properties such as nano-size, porosity, high tensile strength, stability, surface modification ability, and permeability. They can also be useful in terms of physicochemical qualities, being nontoxic and biocompatible, and sensing and responding to environmental changes (Shahriar et al. 2019; Kenry and Lim 2017). The word nano is a Greek term that means “very small,” and fibers are structures that are longer in diameter than they are wide. As a result, nanofibers are structures that are smaller in diameter and have dimensions less than 100 nm when compared to normal fibers (Ramalingam and Ramakrishna 2017). Nanofibers have numerous applications, including health care (e.g., wound dressing, drug delivery, regenerative medicine tissue engineering), energy storage, and other environmental applications (Kenry and Lim 2017). Nanomembranes are quasi-2D structures with a macroscopic surface area and thicknesses that range from 10 to a few hundred nanometers. A freestanding or free-floating self-supported structure (Llorens et al. 2013) with a thickness of 1–100 nm and an aspect ratio of thickness and size greater than 10^6 is classified as a nanomembrane (Pérez-Madrigal et al. 2015). These had a variety of peculiar features that made them valuable in energy harvesting, optics, sensing, plasmonics, actuators, and other biomedical uses (Llorens et al. 2013).

Nanofibers and nanomembranes are manufactured from polymers which can be either biopolymers or synthetic or a blend of both polymers. But biopolymers have low immunogenicity, better biocompatibility, nontoxicity, biodegradability, etc. (Hu et al. 2014). So, biopolymers are used to fabricate nanofibers and nanomembranes for various medicinal and mechanical applications. Both biopolymer nanofibers and nanomembranes can be produced by various types of techniques, but electrospinning has recently attracted a lot of interest, owing to its cost-effective

setup. It has proven to be an efficient, easy, and versatile technology (Subbiah et al. 2005). This chapter provides an overview of the fabrication of biopolymer nanofibers and nanomembranes. The biopolymers that are used to make these nanofibers and membranes and their wide range of applications in different domains are also discussed.

Biopolymer-Based Nanofibers

Biopolymer nanofibers (BPNFs) are the natural polymeric materials that are produced by naturally occurring substances such as plants, animals, bacteria, fungi, shells or wood, etc. (Jain et al. 2020; Bai et al. 2021). Protein and polysaccharide nanofibers are the two most prominent types of biopolymer nanofibers on earth. Polysaccharide nanofibers include chitin fibers (Ifuku and Saimoto 2012) and cellulose fibers (Chen et al. 2018; Zhou et al. 2019) which contain intricate structural patterns and are employed to sustain living organisms' bodies and ensure their existence. Few of the protein nanofibers are found within organisms like keratin and collagen nanofibers (Yang et al. 2019); however, others include silk nanofibers which are produced by spiders and silkworms (Jin and Kaplan 2003).

BPNFs with varying structures and performances are produced by diverse manufacturing processes and raw materials. These nanofibers have captured the attention of scientists, because of their inherent nanostructures and capabilities, and have been extensively used in the energy, material, biological, and environmental domains (Ling et al. 2018). Nanofibers possess effective potential in various medical applications like drug delivery, tissue engineering, wound healing, and other medical devices because of their unique properties like large surface-to-volume ratio, flexibility, biocompatibility, excellent mechanical strength, high porosity, etc. (Jain et al. 2020).

Various techniques for the production of biopolymer nanofibers have been developed recently. Different types of BPNFs with varied morphologies, widths, lengths, degrees of nanofibrillation, crystalline structures, surface chemistries, and chemical constituents are fabricated through different sources using different strategies (Bai et al. 2021). The preparation methods of nanofiber include drawing, self-assembly, centrifugal spinning, template synthesis, phase separation, and electrospinning (Almetwally et al. 2017). But electrospinning is a highly acceptable and widely used method as compared to other techniques as shown in Table 1. This is because electrospinning is a cost-effective, simple, reproducible, continuous, and scalable process that has the property to manufacture continuous biopolymer nanofibers. It is flexible enough to control the dimensions of nanofibers based on shape, size, diameter, porosity, and surface area. The principle electrospinning process is based on the electrostatic fabrication of nanofibers (Yadav et al. 2019; Bognitzki et al. 2001). In 1934, Formhals was the one who published the first patent for the preparation of nanofibers by using the electrospinning technique (Jain et al. 2020). Electrospinning uses a high-voltage electromagnetic field to create electrically charged jets by polymeric solutions or melts, which on drying produces nanofibers when the solvent is evaporated. The more charged fibers are fields directed toward an

Table 1 Comparison between different techniques for nanofiber fabrication

Methods	Scalability	Control of nanofiber morphology	Merits	Demerits	Ref.
Template synthesis	No	Yes	Nanofibers with different diameters are obtained using various types of templates	Organic solvents are required Nonuniform distribution of size Problems of interconnectivity	Liang et al. (2012)
Self-assembly	No	No	Operated in water usually Bioactive functionality	Complex manufacturing process High cost Low mechanical characteristics	Liu et al. (1999) and Yan et al. (2001)
Phase separation	No	No	Minimum apparatus is required for operations Ideally suitable to direct fabrication Used in broad-range fabrication of biomaterials	Organic solvents are necessary Skinning effect on the morphology of scaffolds	Ma and Zhang (1999)
Electrospinning	Yes	Yes	A broad range of various polymers is used Efficient biodegradable and biocompatible materials	Cost-effective Organic solvents are required sometimes Jet instability	Doshi and Reneker (1995) and Agarwal et al. (2009)

oppositely charged collector, which can be either a flat surface or a revolving drum to collect nanofibers. The fiber is subjected to a collection of gravitational, rheological, aerodynamic, tensile, and inertial forces in traditional spinning procedures. The spinning of fibers in electrospinning is principally accomplished from tensile stresses produced in the axial way of the polymer flow through generated charges in the applied electric field (Subbiah et al. 2005).

Applications of Biopolymer Nanofibers

Biopolymers are considered biodegradable substances because of their natural decomposition in the body due to physical environments. So, the requirement for their mitigation is removed. These biopolymers have biocompatibility and no toxicity. They can be made up into nanofibers due to these properties and can be utilized for various functions like drug delivery, tissue engineering, vascular graft implantation, wound healing dressings, biosensors, etc. (Fig. 1). The different merits and applications of the biopolymer-based nanofibers fabricated by the electrospinning technique are summarized in Table 2 (Jain et al. 2020; Hosseini Ravandi et al. 2013).

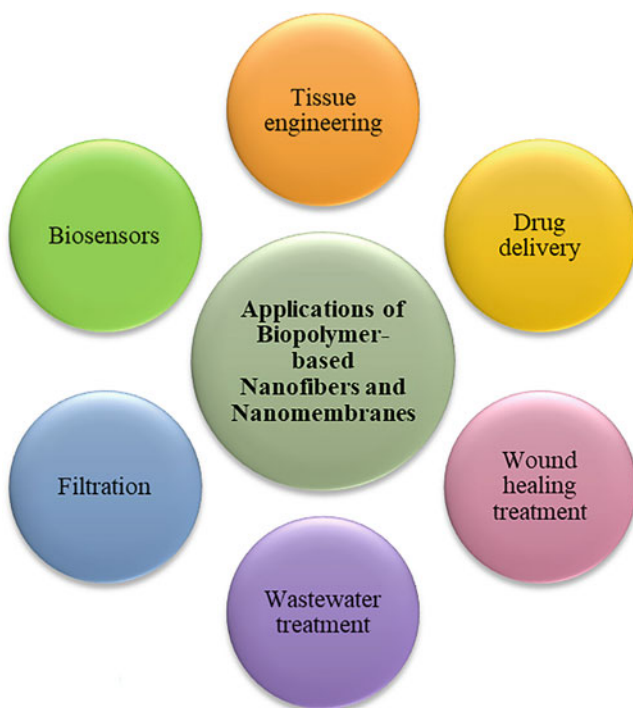


Fig. 1 Various applications of biopolymer-based nanofibers and nanomembranes

Table 2 Different biopolymers used for nanofiber fabrication using electrospinning and their applications

Biopolymers	Source	Carrier polymer	Solvents	Advantages of biopolymer	Applications	Ref.
Chitosan	Fungi and arthropods	PVA PEO	Formic acid Acetic acid	Less toxic, biocompatible, biodegradable	Wound healing, tissue engineering, drug delivery, medical devices, cosmetics, antimicrobial activity	Jayakumar et al. (2010), Ohkawa et al. (2004), Liu et al. (2014) and Duan et al. (2004)
Alginate	Brown seaweed	PVA PEO	Deionized water Deionized water	Low cost, biocompatible, biodegradable, nontoxic	Drug delivery, wound healing, tissue engineering	Schiffman and Schauer (2008), Bhattarai et al. (2006), Tang et al. (2019) and Aadil et al. (2018)
Kefiran	Microorganisms in kefir grains	–	Water	Safety, biocompatibility, biodegradability, emulsifier effect, stabilizing effect	Drug delivery system	Moradi and Kalanpour (2019), Esnaashari et al. (2014) and Dadashi et al. (2019)
Soy protein	Soybean	PEO PVA	Water Water	Low cost, biodegradable, biocompatible	Scaffolds in tissue regeneration	Shankar et al. (2013), Xu et al. (2012), Ramji and Shah (2014), Cho et al. (2010)
Hyaluronic acid	Connective tissues	PVA Gelatin	Water DMF	Biocompatible	Tissue scaffolds, drug delivery	Um et al. (2004), Séon-Lutz et al. (2019) and Li et al. (2006)
Pullulan	Fungi – <i>Aureobasidium pullulans</i>	Gelatin Chitosan	Distilled water Acetic acid	Nontoxic, water soluble, biodegradable	Drug carrier, tissue engineering, scaffolds	Qin et al. (2019) and Sun et al. (2013)
Zein	Corn/maize	–	Water or ethanol	Biocompatible, thermally resistant,	Drug delivery wound dressings	Zhang et al. (2015) and Ansari et al. (2019)
Polyglutamic acid	<i>Bacillus subtilis</i>	–	Trifluoroacetic acid	Nontoxic, biocompatible, biodegradable	Drug delivery, tissue engineering, wound dressings, biodegradable packaging material	Sofi et al. (2019)

Biopolymer Nanofibers in Tissue Engineering Application

Tissue engineering is a multidisciplinary scientific and medical field that emphasizes the rebuilding and regeneration of injured tissues caused by illness, aging, or trauma. The extracellular matrix (ECM) and its function in diverse cell activities like proliferation and differentiation have recently gotten a lot of attention. The natural ECM is made up of fibrous interwoven structural proteins mainly collagen that forms a 3D network with a size of tens of thousands of nanometers (Nathanael and Oh 2021). Electrospinning is considered an efficient method in the tissue engineering discipline to fabricate biopolymer-based nanofibers with a structure related to ECM because of their flexible functional capability.

Because of its prevalence in the ECM, non-immunogenicity, and ease of separation from several sources, collagen has been used in a range of tissue engineering applications. But many of the biological and structural features of this natural scaffolding may be compromised by the normal processes required to separate and reprocess it into a manufactured material (Barnes et al. 2007). The collagen nanofibers have many important features which are useful for tissue engineering, i.e., they can release stress, avoid premature mechanical failure, scatter energy, and allow signals to reach neighboring cells that control functional responses in the body. Moreover, they have high water affinity, excellent biocompatibility, less antigenicity, and the capability to enhance the regeneration of tissues (Koláčná et al. 2007). All of these properties make collagen the deal biopolymer material for tissue engineering.

Electrospinning technology can manufacture collagen fibers that nearly resemble, and at some point may entirely reproduce, the biological and structural features of collagen ECM. The fibers of collagen type I showed a linear relation between fiber diameter and polymer solution when dissolved in hexafluoro-2-propanol (HFP) that ranged from 0.03 to 0.10 g/mL concentration and resulted in the formation of scaffolds which are made of fibers from 100 nm to 5 μ m in diameter (Matthews et al. 2002), whereas the fibers of collagen type III also exhibited a linear relation for collagen concentration ranging between 20 and 90 mg/mL and increased nanofiber diameter (115–612 nm).

Elastin is also gaining attention as one of the structural units of vascular extracellular matrix in the field of tissue engineering. It was studied that based on delivery rate, the human recombinant tropoelastin can be fabricated to nanofibers as well as microfibers by electrospinning (Li et al. 2005). The nanofibers of gelatin have also been lubricated by the electrospinning method using different solvents like formic acid, acetic acid, water, HFP, and ethanol. The gelatin scaffolds and gelatin with synthetic polymer scaffolds have been gaining popularity as tissue engineering scaffolds, with multiple studies demonstrating their effectiveness (Sell et al. 2009). The fibrinogen nanofibers have also been electrospun (Cigané et al. 2021) and have been used as a tissue engineering scaffold with effectiveness (Wnek et al. 2003). Chitin along with polyglycolic acid (PGA) has been fabricated into biocompatible and biodegradable nano-scaffolds for tissue engineering applications. The polymer PGA was used due to its biocompatibility and biodegradability. The diameter of these nanofibers is distributed between 50 and 350 nm, with an average diameter range of about 140 nm (Park et al. 2006).

Biopolymer Nanofibers in Drug Delivery

One of the most promising applications among the potential ones is drug delivery. A drug delivery system is utilized to increase the therapeutic effects of a pharmaceutical drug or a natural substance in a specific diseased region while minimizing the toxicological side effects. Nanotechnology is a multidisciplinary science that enables the synthesis of polymers at the nanoscale scale for a variety of medicinal purposes. The biopolymers show biodegradability, biocompatibility, and minimal immunogenicity in drug delivery as compared to synthetic ones (Jacob et al. 2018).

Electrospinning for drug delivery is interesting because of its excellent efficiency of encapsulation, low cost, ease of operation, greater loading capacity, and synchronized transfer of many treatments (Wang et al. 2010). To reduce toxicity and increase the therapeutic impact of standard dosage methods, many delivery strategies have been developed. Nanofibers have gotten a lot of interest in the last 10 years. Electrospun fibers were created using natural polymers. These biopolymers possess effective antimicrobial properties, good clinical functionality, less immunogenicity, and better biocompatibility. Natural polymers mostly include polysaccharides (chitosan, cellulose, dextrose, etc.), proteins (collagen, silk, gelatin, etc.), and a few biopolymer composites and derivatives (Schiffman and Schauer 2008). The properties of different polysaccharides, like chitosan, starch, cellulose, hyaluronic acid, dextran, chitin, alginate, etc., have been described which can be used for different biomedical uses like drug delivery, dressings of wounds, and enzyme immobilization (Lee et al. 2009).

Due to the polycationic nature of chitosan, they exhibited better anticancer activities. In vitro anticancer efficacy of quaternized chitosan derivatives against HeLa, Hep3B, and SW480 cells is well recognized (Toshkova et al. 2010). Chitosan is coupled with polylactic co-glycolic acid (PLGA) and polyethylene glycol (PEO) to create nanofibers that have been used as delivery methods for *Trichoderma viride* (Spasova et al. 2011) and *Bacillus* sp. (Zupančič et al. 2018).

Alginate and its derivatives are biodegradable and have controlled porosity, yet pure sodium alginate electrospinning is problematic. The use of another polymer is compulsory to control the viscosity and spinnability of alginate. Sodium alginate, in combination with PVA, has been electrospun into nanofibers that have worked as insulin delivery devices (Sharma et al. 2013). Fish sarcoplasmic proteins, as well as elastin silk, and collagen are the most used electrospun proteins for drug delivery applications. The sarcoplasmic proteins of Atlantic cod are elongated polymers with fiber diameters ranging from hundreds of nanometers to some micrometers. These properties, along with the potential health benefits of eating fish, make fish sarcoplasmic proteins appealing materials for electrospinning of nanofibers. As a result, these nanofibers can be employed to deliver antidiabetic medications like insulin (Stephansen et al. 2015). Some spiders, worms, and insects manufacture silk proteins, which are a distinctive family of naturally occurring fibrous biopolymers. Because of their self-assembly, controlled shape and morphology, gentle biodegradability, and biocompatibility, they are attractive materials for drug administration and tissue engineering applications (Elzoghby et al. 2015).

Biopolymer Nanofibers in Wound Healing Treatment

The wound dressings manufactured by electrospun nanofibers have excellent properties to speed up the process of healing. Their three-dimensional structure mimics the extracellular matrix architecture found in the skin, which performs a significant function in proliferation and cell adhesion (Miguel et al. 2019a). The porous structure of the nanofibrillary matrices is compatible with nutrient and gaseous exchanges, adsorption of injury exudates, and bacterial contamination prevention so that the architecture of the membrane will aid proliferation, differentiation, and adhesion of cells (Yuan et al. 2018).

Biopolymer nanofibers produced using a versatile electrospinning technology have some advantages, including efficient biodegradability, nontoxicity, ease of operation, effective biocompatibility, and even antibacterial activity while using chitosan (Ambekar and Kandasubramanian 2019). When employed in the formulation of electrospun nanofibers as wound dressings, biopolymeric macromolecules produced from polysaccharides have been shown to improve wound healing. The biomaterials based on polysaccharides from seaweed, specially alginates, have gotten much attention in the area of wound healing applications due to their cost-effectiveness, versatility, and abundance (Hao et al. 2020). The salts of chitosan nanofibers have been found to suppress the growth of *S. aureus* bacteria in the existence of very low fiber quantities. The negatively charged residues of macromolecules at the cell membrane surface are thought to interfere with the polycationic character of migrating glucosamine portions of chitosan, resulting in the death of microorganisms. As a result of their biodegradability and peculiar features that could help manage microbes, composite materials containing chitosan fibers can be used considerably in more applications (Torres-Giner 2011).

Due to its nontoxicity, antimicrobial activity, biodegradability, and biocompatibility, chitosan is one of the significant biopolymers for the manufacture of electrospun nanofibers. The physical, chemical, and ionic cross-linking techniques can be used to cross-link electrospun chitosan nanofibers with aligned or random fiber structures. The cross-linked electrospun chitosan nanofibrous membranes had better stability, biocompatibility, and mechanical properties, all of which are important for wound healing and other applications as shown in Fig. 2 (Cui et al. 2021).

Scientists are interested in plant-derived polysaccharides because of their biological properties like antioxidant, antidiabetic, and anticoagulant, as well as key traits such as biodegradability, nontoxicity, and environmental compatibility (Ghliissi et al. 2020). Plants are one of the most important suppliers of polysaccharides in terms of resources. Furthermore, their exceptional biological properties and inexpensive manufacturing costs make them suitable for wound healing treatment.

The starch-based nanofibers produced by electrospinning have a high porosity and a large surface area; also, they are biodegradable, biocompatible, and bioresorbable. As a result, these starch nanofibers have a lot of potential in the pharmaceutical industry, including tissue engineering and wound healing (Liu et al. 2017). In the area of the development of wound dressing, pectin, chitosan, and alginate are the most widely used ionic polysaccharides. The various molecular structures of these pectins

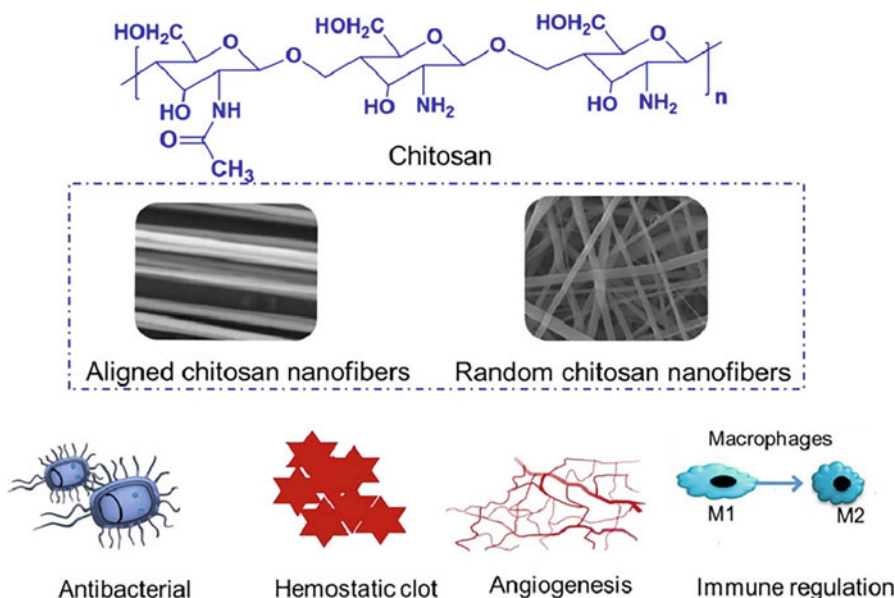


Fig. 2 Chitosan nanofibers for wound healing applications. (Reprinted from Cui et al. (2021) with the permission of (KeAi Publishing Ltd., China))

result in different properties of their nano-sized dressings, which are suitable for a variety of biomedical applications (Mogoşanu and Grumezescu 2014).

Chitosan is a natural substance that has been shown to have an important role in wound healing because of its unique properties, i.e., increasing hemostatic and analgesic effects, activating neutrophils, and better interaction with phospholipid membrane molecules, extracellular matrix formation, and activation of macrophage (Venkataprasanna et al. 2020). Chitosan and arginine were electrospun together to create hydrophilic and good porous wound healing dressings that copy the qualities of the natural skin. In contact with these membranes, human fibroblasts attached and multiplied, indicating biocompatibility. Bacteriostatic activity against *Staphylococcus aureus* and *Escherichia coli* was also found in these nanofiber mats. In comparison with pure chitosan nanofibers, in vivo administration of these nanofiber mats on Wistar rats revealed better wound healing and regeneration of tissues (Wani et al. 2017).

Biopolymer Nanofibers as Biosensors and Analytical Systems

Many disciplines rely on the identification of biological molecules at low quantities, which necessitates high sensitivity. For these applications, highly conducting biosensors are now being developed. They are often made of biofunctional materials and have a high level of sensitivity and selectivity. These biosensors are used in a variety of applications, including the environment, food, and medicine (Rezaei et al. 2015).

Nanofibers permit enzymes to be immobilized and can be developed into sensitive electrochemical biosensors due to their large surface area, porous nature, and spatial structure. By immobilizing genetically modified acetylcholinesterase inside electrospun chitosan and PVA nanofibers, a highly sensitive electrochemical biosensor was effectively produced. Then, this enzyme-immobilized system was effectively designed to detect pirimiphos-methyl in olive oil. The biosensor functioning for detection of pirimiphos-methyl enhanced to a low value of detection limit which is 0.2 nM as compared to already internationally existing defined value which is 164 nM (El-Moghazy et al. 2016). The Hg^{2+} ions may be detected in various analytical samples using fluorescence sensor systems which are composed of rhodamine B-adorned chitosan-based nanofibers. When the spirolactam ring of the rhodamine unit is opened, these rhodamine-based chitosan nanofibers permitted the particular detection of Hg^{2+} ions. These mats are improved as sensitive sensing probes due to the large specific surface area provided by nanofibers. This strategic formation offers the ability to arrange and build good functioning sensing materials for the environmental identification of multiple metal elements (Wani et al. 2017). By grafting DNA on cellulose nanofiber, a double-function cellulose nanofiber-based bionic biosensor with effective biocompatibility was produced for detecting silver ions (Ag^+) and acetylcholinesterase (AChE). These silver ions served as recognition sites for the detection of AChE (Wang et al. 2021).

Natural polymer nanofibers are used in chromatographic analyses as well. Electrospinning was used to make chitosan-iron nanofiber composites, which were used to extract a small quantity of 9-tetrahydrocannabinol from blood samples after it was detected by ultraviolet detectors. The substantial surface area of these natural nanofiber composites showed that an analyte and adsorbent had a robust contact. This innovative sorbent might provide a higher analytical role in terms of detection limit, repeatability, and recovery while using less toxic organic solvents and sorbent materials than the adsorbent in C18 columns (Wani et al. 2017).

Filtration

Nanofibers can be used as filters to capture small particles. Generally, filter efficiency is improved linearly by decreasing the thickness of the filter membrane and increasing the applied pressure. Electrospinning is the technique that can lessen filter diameters to smaller than 0.5 μm , which results in enhanced filter efficiency when pressure is increased (Rezaei et al. 2015). When regenerated cellulose nanofibers are used which are formed from the alkaline analysis of nanofibers of cellulose acetate with diameters ranging from 200 nm to 1 μm , then a filter membrane was created. The nanofibers were heated for 1 hour at 208 $^{\circ}\text{C}$ to increase their mechanical characteristics and structural stability. In order to isolate biomolecules such as bilirubin and bovine serum albumin, a filter membrane was surface revolutionized with Cibacron Blue F3GA. Furthermore, when compared to conventional microporous membranes, this filter membrane had a better water permeability (Ma et al. 2005). Similarly, the affinity membrane was produced from a regenerated cellulose nanofiber. This membrane was functionalized with protein A/G and showed a high

affinity for immunoglobulin G (IgG) which makes it applicable for the purification of IgG (Ma and Ramakrishna 2008).

Another composite membrane was produced whose top layer was made of cellulosic nanofibers, the middle layer was made of a polyacrylonitrile electrospun scaffold, and the supporting substrate was made of polyethylene terephthalate nonwoven. When compared to the identical membranes without cellulose-based nanofibers, the efficiency of this filter membrane rose by nearly five times (Ma et al. 2014).

The chitosan-based nanofibers also exhibit desirable filtering features. It was employed in a huge range of filtration uses, including purification of water and air filtration. The blend solutions of chitosan and PEO are electrospun onto a nonwoven polypropylene substrate to create a nanofibrous filter medium. They proved the effectiveness of filter media of chitosan-based nanofibers for the mitigation of pathogenic microorganisms, contaminated particle media, and heavy metal ions from air and water. The antimicrobial, heavy metal binding, and physical filtration effectiveness of chitosan-based filter media were investigated and connected with the physical features and surface chemicals of nanofibrous filter media (Jayakumar et al. 2010; Desai et al. 2009). The metal adsorption of chitosan electrospun nanofiber mats of diameter 235 nm in aqueous environments was investigated. These nanofiber mats counterbalanced with potassium carbonate in water have demonstrated better stability of water erosion and greater adsorption affinity for metal ions (Haider and Park 2009).

Biopolymer-Based Nanomembranes

Nanomembranes (NMs) are the primary building block of probably all living creatures – life as we know it would not be conceivable (Jakšić and Jakšić 2020). NMs establish nowadays fascinating matter thus in the broad area of nanotechnology. They fit the definition of self-sufficient, independent, or free-floating freestanding architect whose length-to-width and also breadth-to-wideness characteristic proportions both overdo 100 nm (Jiang et al. 2004). A class of intriguing nanomaterials includes nanomembranes and nanosheets, which are anticipated to offer sophisticated industrial applications in addition to fundamental scientific advancements (Watanabe et al. 2009). Due to their recyclability, high-performance and functional bio-nanocomposites are attractive examples of biologically generated materials well with hierarchical management platform because of their flexibility, bioactivity, biocompatibility, and availability of multiple active sites on the surface for adding unique functionalities (Xiong et al. 2018). Biopolymers are covalently linked long chains of monomeric units that make up polymeric biomolecules. The three main categories of biopolymers are polynucleotides, polypeptides, and polysaccharides. Due to their biocompatibility, biodegradability, and processability, biopolymers are hypothetical synthetic biomolecules that might be used in a range of industrial fields (Jayan et al. 2021).

Because of their exceptional mechanical tenacity, effective stacking transmission, and unique flexible properties, extremely thin nanomembranes possess the capability to be used in place of a variety of elastic useful devices (Shi et al. 2014). By executing a bulging test and an AFM (*atomic force microscope*) bending experiment, the flexibility characteristics of reduced graphene-oxide cellulose nanocrystal nanomembranes were explored. The results reveal better interfacial contacts that improve the robustness and elasticity of reduced graphene oxide-cellulose nanocrystal nanomembranes (Kim et al. 2016). These characteristic nanomembranes are made of biopolymers that have several uses. Different nanomembranes can be created using a variety of approaches. Among the important strategies are surface modification, nanotechnology, nanofabrication, layer-by-layer deposition, chemical vapor deposition, 3D printing, sol-gel processing, and others (Ciganè et al. 2021). The other techniques include wet-phase transposition (Chen et al. 2011), dry/wet-phase transposition (Khan et al. 2020), electrospinning method (Jin et al. 2019; Miguel et al. 2018), etc.

Applications of Biopolymer Nanomembranes

A focus of the analysis is on engineering and biomedical applications (Albisa et al. 2017), such as supercapacitors (Bhushan et al. 2014), solar cells and batteries (Anjaneyulu et al. 2005), separation technologies and wastewater treatment (Benson et al. 2019), catalytic sensing (Albisa et al. 2017), wound dressing (Ariyoshi et al. 2021), cancer therapy (Ariyoshi et al. 2021), tissue engineering (Aliyu et al. 2018), and drug delivery (Albisa et al. 2017). These systems have definite benefits, such as positive impacts on post-injury recovery (such as the restoration of injured cerebral, vertebral, and periphery nerves, tendons and ligaments, and epidermis) and no lasting fitness threats.

Molecules' Selective Separation

Membrane-based segregation of nanomaterials and macromolecules such as proteins and polypeptides is becoming more common because it is an inexpensive and scaled-up technique. Trimethylsilyl cellulose (TMSC) was regenerated to create a self-supporting cellulose membrane with pore dimensions reaching from 1.5 to 3.5 nm that help the separation of tiny molecules based on size and charge and exhibit excellent anionic molecule differentiation (Puspasari et al. 2016). By combining cellulose nanofibers with poly(dimethylsiloxane) (PDMS), robust, nanometer-thick semipermeable membranes were created (CNF). Spin coating is a repetitive process that produces multilayered nanocomposites. Through a fault-free PDMS sheet of almost 17 nm width, a durable PDMS-CNF nanomembrane provides exceptional penetration of 50,000 GPU. The asymmetrical configuration of PDMS and CNF had an impact on the nanomembrane's capacity for gas permeation; the CNF layer's sides showed reduced gas permeability, while the PDMS composite layer functioned as a direct air-capturing medium (Ariyoshi et al. 2021).

Wound Dressing

Wound healing is a well-defined yet intricate chain of processes generally consisting of five overlapping steps: hemostasis, inflammation, migration, cell growth coupled with matrix healing, and epithelialization coupled with modification of the scar tissue (Maver et al. 2015; Boateng et al. 2008). Chitosan membrane was manufactured by utilizing techniques of dry/wet-phase inversion and sterilized with ethylene dioxide and gamma radiation and characterized regarding thickness, tensile strain, morphology, strain break percentage, and Vero cell cytotoxicity in vitro that was not affected, hence appropriate for wound dressing material (Marreco et al. 2004). Fish scale collagen peptides are combined with chitosan oligosaccharide to form fish scale collagen peptides/chitosan oligosaccharide nano-fibrous membranes (FSCP/COS-NFM), and fiber-forming capacity is increased by using polyvinyl alcohol through electrospinning technique. FSCP/COS-NFM exhibited antimicrobial efficacy toward *Escherichia coli* and *Staphylococcus aureus*. It supported the proliferation of human skin fibroblasts and was used in wound dressing (Wang et al. 2011; Jayakumar et al. 2011). Chitosan-based membranes were synthesized through immersion-precipitation phase inversion using a casting method by using the acetic acid aqueous solution as solvent. It showed strong inhibition to bacteria penetration of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It demonstrated outstanding oxygen permeability, restricted evaporation, and enhanced fluid drainage capacity. Chitosan membrane acted as a hemostatic agent and stopped bleeding when applied to the wound. On the 35th perioperative day, the wound's collagen fiber deposits were neatly structured and aligned along one axis parallel to the skin's surface, indicating that the injured tissue had been perfectly repaired. The outcome suggested that the chitosan membrane is asymmetrical and may have an application as potential material for wound dressing as shown in Fig. 3 (Mi et al. 2001).

To meet the conditions (oxygen permittivity, regulated evaporation phenomenon, and the discharge of injury metabolic byproducts) is required for such membrane to be employed as a wound bandage; an AgSD-assimilated chitosan membrane along

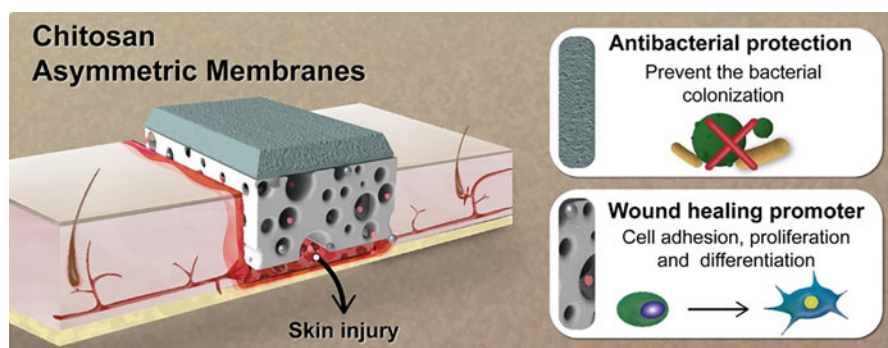


Fig. 3 Demonstration of the major characteristics of asymmetrical membranes used to enhance the healing of wounds. (Reprinted from Miguel et al. (2019b) with the permission of Elsevier)

with constant antibacterial efficiency has been generated through a dry/wet-stage inversion process. The asymmetrical chitosan membrane contains AgSD, which works like a rate-controlling wound bandaging to release sulfadiazine and silver ions during the time (Mi et al. 2003). A unique thermo-responsive hydrogel membrane consisting of poloxamer 407, sodium alginate, platonic F-127, and polyvinyl alcohol (PVA) was developed to speed up the wound healing process. The created hydrogel membranes had exceptional inflammation capabilities, surface permeability for sustained drug release, and strong mechanical qualities as well as ultimate tensile to endure external frictional forces despite sealing the injury (amikacin). According to antibacterial data, *S. aureus* and *P. aeruginosa* were significantly more inhibited by amikacin-loaded hydrogel membranes. Our innovative alginate-based hydrogel membrane showed great promise in the rigorous assessments as an effective wound healer for quicker wound healing (Abbasi et al. 2020). Alginate-based mats with or without zinc oxide nanocomposites were electrospun and afterward subjected to a rinsing process to develop exceptionally stable products with thinner, homogenous nanofibers with 100–30 nm diameter. Along with the easiest and harmless manufacturing process, except for often used animal collagen-extracted methods, the suggested alginate-based solutions could result in safe and economical surgical coverings as well as wound covering membranes (Doderio et al. 2019).

Tissue Engineering

Transmittance, bidirectional oxidation, redox stabilization, bioactivity, lipophilicity, 3D structure, and surface morphology are a few of the desirable characteristics for tissue engineering applications (Ramalingama and Tiwari 2010). The carboxymethyl chitin (CM-chitin), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) reagent, and 2-aminoethane sulfonic acid were combined to create sulfated chitin. The biospecific breakdown of the sulfated chitin has intriguing implications for tissue engineering and medication delivery (Jayakumar et al. 2008). In bone tissue engineering applications, biomaterials play a crucial role since they can replicate the structure and chemical composition of bone tissues at the nanoscale level. In particular, natural polymer composites with chitin, chitosan, or collagen are preferable because they exhibit properties like biocompatibility and biodegradability that are necessary for bone tissue engineering (Li et al. 2014).

Wastewater Treatment

Wastewater from most home and industrial processes contains undesired harmful pollutants. Many physical, chemical, and biological technologies have been reported over the previous three decades, including flotation (Afzal et al. 2019), precipitation (Mavhungu et al. 2021), oxidation (Li et al. 2021; Deng and Zhao 2015), solvent extraction (Hall et al. 1990), evaporation (Sharshir et al. 2020), carbon adsorption (Chai et al. 2021), ion exchange (Kansara et al. 2016), membrane filtration (Hube et al. 2020), electrochemistry (Smith 1972), biodegradation (Yu et al. 2020), and phytoremediation (Hu et al. 2020), but here our concern is biopolymer-based nanomembrane filtration. Because of its great effectiveness and lesser energy

utilization as compared to distillation processes, membrane desalination technology is widely seen as a solution to the problem of water scarcity (Aliyu et al. 2018). Cellulose from agricultural waste is mixed with chemicals to form two biopolymer-based nanomembranes (ramie and sisal cellulose nanomembranes) of varying pores ranging from 120 to 230 nm and from 20 to 80 nm, with thermal decomposition at 230 and 450 °C, pH range from 6.7 to 7.2, and TDS (total dissolved solids) of 368 and 523 mg/L, respectively; by using these nanomembranes, we can purify the wastewater (Hassan 2020). ZIF-8@CM composite membranes were made by synthesizing ZIF-8 in situ on a cellulose matrix using an alkali/urea method. This membrane might be designed for the purification or filtration of dyes, and it has been shown to have high failure rates for rhodamine B and good renewability, providing the potential for a useful application in the treatment of wastewater (Chen et al. 2020). The state inversion approach was employed to create the plane membranes by combining cellulose acetate with polyethylene glycol (additive) and N-methyl-2-pyrrolidone as solvent. Additionally, membrane's pure water penetration flux was examined (Mohammadi and Saljoughi 2009). The electrospinning technique is used to create alginate-based membranes that contain ZnO NP (nanoparticles), which were then used as biosorptive constituents. The membranes have a homogeneous structure that is made up of linked cavities that are an average size of 140 nm and nanofibers having a diameter of 100 nm on average. UV-vis spectrophotometric studies were carried out to assess membrane uptake/release capabilities using Congo red (CR) and methylene blue (MB) dye solutions in water, which were selected equally as ideal probes for basic or acidic categories, respectively. Additionally, after a few quick washing procedures in deionized water, good reusability was attained for MB. Surprisingly, desorption efficacy under physiologically analogous circumstances is designated quite great for MB but lowered for CR, showing ionic associations have an impact on the release process. The outcomes indicate that the electrospun membranes were employed in drug supply and decontamination processes (Doderio et al. 2020). Figure 4 portrays the wastewater treatment through membrane filtration.

Drug Delivery

The term “drug delivery systems” (DDS) refers to several different drug carriers that are used to carry medications to specific muscles, organs, subcellular organs, and cells for drug discharge or absorption (Li et al. 2019). The technique and route by which an active pharmaceutical ingredient (API) is supplied to increase its desired pharmacological impact and/or convenience and/or to reduce undesirable effects are how drug delivery (DD) is defined. This can be summed up as “improving drug efficacy” (Bae and Park 2020). Bacterial cellulose (BC) is a pure kind of cellulose that is manufactured by several numbers of bacteria that can control how quickly model pharmaceuticals are released through the skin. The positive results of the 24-hour patch test on the skin confirm the potential utility of bacterial cellulose membranes as carriers for transdermal drug delivery (TDS) and topical drug delivery (Almeida et al. 2014). Semipermeable membranes, commonly made up of cellulose acetate (CA) through a variety of flux boosters, regulate the pace at which

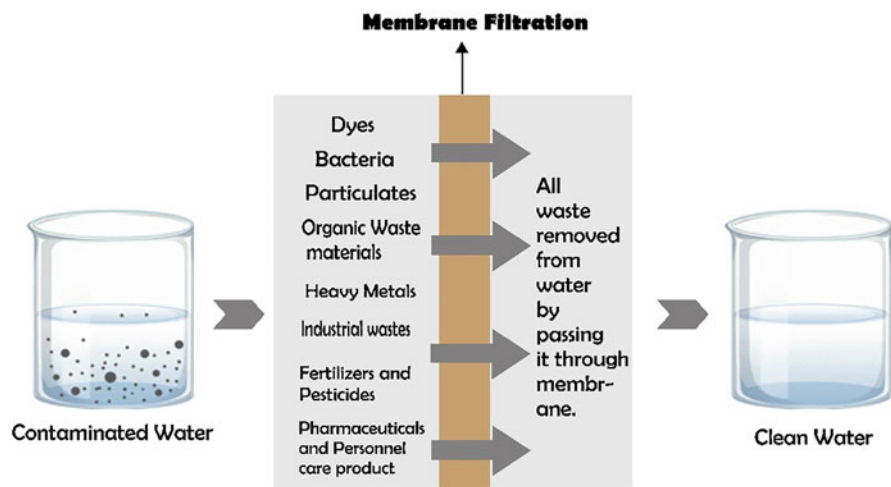


Fig. 4 Wastewater treatment through membrane filtration

medications are released from OROS[®] (orderly discharge osmotic delivery technology of ALZA Corporation). An effective substitute was found to be cellulose acetate butyrate (CAB). The CAB membrane was equally durable as the CA membrane but had greater drying abilities, providing benefits, particularly for compositions that are thermolabile. It has been shown that osmotically regulated drug administration is possible with CAB-based membranes. The examined membranes were as durable as CA membranes, showed high stability, and could have their membrane permeability changed by adjusting the film's thickness and flux enhancer levels (Shanbhag et al. 2007). To create membranes for various biomedical applications, carboxymethyl cellulose (PVA-PEO) and poly(vinyl alcohol)-poly(ethylene oxide) blends were created using two methods: solvent casting and freeze-drying. The porous architecture of the FD membranes, which are three-dimensionally linked, provides routes for fluids to exit the wound. The membranes' overall porosity was discovered to be between 89% and 92%. The WVTR (water vapor transmission rate) for the FD membranes was discovered to be between 2000 and 3000 gm^{-2} per day. Ciprofloxacin hydrochloride, an ideal medication, was as well included in the matrices, and medicine discharge was investigated. A zone of inhibition approach was used to assess the membranes' antibacterial capacity against *E. coli* (Agarwal et al. 2013). The fabrication of four distinct alginate-based membranes, along with other copolymers (e.g., Carbopol, Tween, and polyvinylpyrrolidone) and other elements with various functions, is introduced. Thermogravimetric techniques (TGA) and Fourier transform infrared and ultraviolet spectroscopic techniques, as well as the scanning electron microscope, are utilized to examine membranes and binary blends generated among the elements utilized in membrane production and methotrexate. The transdermal delivery of methotrexate can be accomplished using the A-MTX (sodium alginate glycerol methotrexate) and AGP-MTX (sodium alginate glycerol polyvinyl pyrrolidone methotrexate) membranes (Bajas et al. 2021).

Conclusion

Biopolymer-based nanofibers and nanomembranes due to their recyclability, adaptability, biodegradability, biocompatibility, and accessibility of numerous reactive sites for introducing novel functionalities are appealing representatives of high performances and functions. The domain of synthesizing and fabricating biopolymer-based nanofibers and membranes is so vast that several areas require improvement. Until now, utmost research has been directed toward the development of new synthetic nanofibers and nanomembranes for effective wound healing materials that mimic the extracellular matrix architects present in the epidermis. They have definite benefits, such as positive impacts on wound healing such as repair of damaged spinal, cranial, and peripheral nerves, connective tissues, and skin and have no long-term health risks. Currently, scientists are attempting to construct NFs and NMs from biopolymers to reduce the toxic effects of chemicals considering their effective use in wastewater treatment due to their antimicrobial properties, tensile strength, biodegradability, and recyclability. All categories of NFs and NMs have their peculiar functionalities and significance accompanied by certain limits. To eliminate these shortcomings, diverse biopolymers are now introduced as promoters to NMs and NFs and are usually favored in medical application like tissue engineering, drug delivery, wound healing and dressing, sensors, and purification as well as filtration. There must be a need to devise more appropriate methods to enhance the effectiveness of these NFs and NMs which can be organized by introducing biopolymers that improve various properties to be used in biomedical applications. They should necessarily be facile, nontoxic, cost-effective, efficient, and excellent materials, and their exposure to the human body must not be harmful. Most of the recent research has been done on chitosan- and cellulose-based NFs and NMs, and researchers need to be focused on producing other biopolymer-based nanofibers and nanomembranes which must be efficient for various medical and nonmedical applications.

Cross-References

► [Nanofibers and Nanomembranes of Biopolymers](#)

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Amylose–Amylopectin Ratio

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Comprehensive Understanding of Structure, Physicochemical Attributes, and Applications of Starch

Sharon Varghese, Monika Awana, Debarati Mondal, M. H. Rubiya, Krishnakumar Melethil, Archana Singh, Veda Krishnan, and Bejoy Thomas

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Abstract

Starch is a relevant biopolymer since it is easily modifiable and can be used as an alternative material to several petrochemical-based nonbiodegradable materials. The physicochemical characteristics and subsequent uses of starch are dependent on their botanical origin, which has a big impact on the granule structure and amylose to amylopectin ratio, which ranges from 15:85 to 35:65, with the

S. Varghese · K. Melethil · B. Thomas (✉)

Department of Chemistry, Newman College, Thodupuzha, Kerala, India

e-mail: bejoy.thomas@newmancollege.ac.in

M. Awana · D. Mondal · A. Singh · V. Krishnan (✉)

Division of Biochemistry, ICAR – Indian Agricultural Research Institute (IARI), New Delhi, India

e-mail: vedakrishnan@iari.res.in; veda.krishnan@icar.gov.in

M. H. Rubiya

Mahatma Gandhi University Centre for Research in Chemistry, Mar Athanasius College, Kothamangalam, Kerala, India

exception of waxy starch and high amylose maize starch. The amylose to amylopectin ratio has a momentous effect on the biochemical, physicochemical, mechanical, and thermal properties of native and modified starch. The chapter provides a comprehensive understanding of the effect of amylose to amylopectin characteristics of starch on their physical and biochemical properties of starch, including the digestibility, dispersibility, rheological, and reinforcing properties.

Keywords

Starch · Amylose/amylopectin ratio · Crystallinity · Rheological properties · Modified starch · Gelatinization · Heat moisture treatment (HMT)

Introduction

A solid polysaccharide with no flavor or color, starch is made up of the components amylose and amylopectin, which are in turn each made up of multiple glucose monomer units. Along with cellulose and glycogen, starch is one of the three main biorenewable natural compounds that may be found on Earth. Despite sharing the same monomer component, glucose, the various stereochemical linkages cause a completely distinct conformation, and as a result, diverse physicochemical characteristics (Al-Assaf et al. 2007). A polysaccharide called starch is made up of glucose molecules linked together by – (1–4) and – (1–6) connections. It is made up of two different types of substances, amylose and amylopectin. Amylose is a linear polymer made up of glucose units connected by -D-(1–4) glycosidic bonds, making up around 20–30% of all starch. Amylopectin, on the other hand, is a branched-chain polymer with around 5% of -D-(1–6) links in the branched chain and -D-(1–4) glycosidic linkages in the linear chain.

Structural differences existing in the starch constituents' deliver starch granules a semi-crystalline structure, where amylose contributes to amorphous and amylopectin contributes to crystalline regions. These semi-crystalline starch granules are highly species-specific (Singh et al. 2008a) and thus have different size, shape, and morphology depending on the biological sources, topology, geographical conditions of the cultivated locations, and cultural practices (Laurentin and Edwards 2013). It can also be affected by environmental factors such as climate, temperature during growth, harvest date, water stress, and storage temperature (Singh et al. 2008b).

According to Robyt (2008), the molecules of amylose and amylopectin generate various degrees of intramolecular and intermolecular hydrogen and hydrophobic bonds that keep the molecules together and produce a granule that is insoluble in water. The process of granule biogenesis involves irregular starch production, which is greatly influenced by both genetic characteristics and environmental factors (Jane 2007). A starch granule's amylose and amylopectin structure varies from the center to the edge (Pan and Jane 2000). Starch appears as a white, soft powder that is insoluble in water but is sometimes seen in a colloidal state that settles over time. Due to its large relative abundance, ease of isolation in a highly pure state, enzymatic hydrolysis to glucose and/or other maltodextrin products, corresponding chemical modification, and ease of solubilization, starch is an important substance. Because

starch is an environmentally acceptable, nonpolluting, and biodegradable material, it has undergone substantial research in the creation of packaging materials to replace dangerous plastics.

Granule Structure of Starch

Granule structure of starch is a microscopic structure that possesses its constituent molecules of amylose, amylopectin, and small amount of water, organized in micelle within a durable and morphologically identifiable form (Imberty et al. 1991). The chains of the amylopectin molecules along with amylose stack on top of each other that can be considered two-dimensional molecules. It gives radially oriented crystallites, which is 9 nm in size. Within the micelle, there are normal chains, chain terminations, and branches. Starch chains that enter and exit the micelle create the amorphous phase of the starch granule (French 1984).

The starch granule, which has an entire morphology dimension (Fig. 1) in the micrometer range, contains a complex hierarchical structure that can be considered on various separate length scales, including molecules (scale factor: 1), lamellae (scale factor: 9 nm), growth rings (scale factor: 100 s nm), etc. To date, little is known about the three-dimensional lamellar and growth ring structure of starch. There are currently two models for the lamellar structure of potato starch. The first model

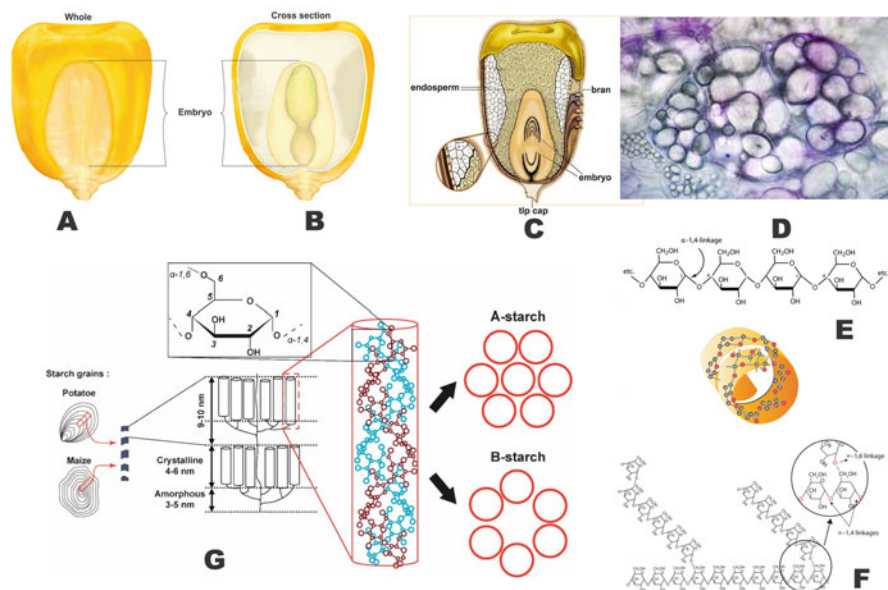


Fig. 1 The starch's hierarchical structural levels of organization, which demonstrate a seed arrangement from cm to m range (a–c). The circles represent the double helices visible from the top of a hypothetical granule with amorphous and semi-crystalline growth rings extending from the hilum (d), amylose component of starch (e), and amylopectin double helices generating the crystalline lamellae of the blocklets (f) (g)

predicts that the lamellae will be twisted into helical structures that converge side by side, and the second model includes helical lamellae that form “blocklets” with restricted lateral coverage. Depending on the source plant, these blocklets have a spherical shape and come in sizes between 20 and 500 nm. A single-crystalline growth ring is thought to consist of two end-to-end blocklets on average (Fig. 1) (Waigh et al. 1999).

Because starch is insoluble in water, its granules are incredibly easy to separate. Granules of starch or amyllum, which are produced by biosynthesis and are composed of densely packed polysaccharides and a little amount of water, are semi-crystalline. Utilizing techniques such as NMR spectroscopy, small-angle X-ray microfocus scattering, X-ray microfocus diffraction, and others, it has been discovered that the natural starch granules’ annular growth ring structure is made up of alternating crystalline and semicrystalline lamellae. The developing rings surround the hilum core and are both crystalline and amorphous (Lin et al. 2011). Crystalline lamellae are formed when amylopectin branches interlock to form double helical structures. Due to its greater interaction with amylose, amylopectin has reduced crystallinity but still predominates in the double helical form in the semi-crystalline lamellae. The starch granule’s multiscale structure is depicted in Fig. 1 and includes (a) the granule itself (2–100 μm), (b) growth rings (120–500 nm), (d) blocklets (20–50 nm) formed of super-helical lamellae (9 nm) both amorphous and crystalline, (c) amylopectin, and (h) amylose chains (0.1–1 nm) (Dufresne 2014; Bertoft 2017).

In most situations, amylose, which is essentially unbranched, accounts for the remaining 25–28% of the starch in cereal grains, while amylopectin, which contains many branched chains, makes up the majority of the starch, accounting for 72–75%. Waxy starches are those that include less than 5% amylose, high-amylose, or amylo-starches are those that contain more than 35% amylose, and normal starches are those that contain 20–35% amylose. (Tester et al. 2004) Amylose, which is thought to be robust in nature, makes up the amorphous region. The crystalline component of the starch granules with a high amylopectin content is easily susceptible to melting at low temperatures because of a disruption in crystallinity (Yuryev et al. 2004).

Starch granule crystallinity is maintained even after amylose has been leached off (Zobel and Stephen 1995). It is obvious that a significant portion of the crystalline growth rings must be formed by amylopectin. According to Imberty and Pérez (1989), the amylopectin branch connections that form the crystallites are clustered and have many exterior chain segments with 15–20 glucose residues entwined into double helices. All chains are arranged in arrays, and the parallel stranded double helical structure is found in pairs. With a pitch of 2.1 nm and six glucose residues on each turn of each strand, two chains bind together to form a double helix. These double helices measure 4–6 nm in length (Bertoft 2017).

Due to differences in their amylose to amylopectin ratios, different sources of starch have varied characteristics. The structural compactness of amylopectin, which is generated in endosperm during grain formation, determines the size and shape of the starch granule. The shape of amylopectin is determined by the ratio of long-chain to short-chain branches, which also affects the starch compactness and starch granule size (Jane 2007). The amount of phospholipid, phosphate-monoester, lipids, granular

architecture, distribution of branched chains in amylopectin, amount of amylose, and crystalline structure all have a significant impact on the starch's functional qualities (Singh and Kaur 2004; Singh et al. 2003). The starch granules can be as small as a few sub-microns to as large as 100 μ m, and they can be spherical, lenticular, oval, elliptical, disc-shaped, etc. (Zhou et al. 2013). Additionally, they can be found as flat thin plates, irregularly formed polygons, and circular pancake shapes with different thicknesses (Jane et al. 1994). Some of the granules' surfaces include tiny holes or apertures, and the degree of porosity varies correspondingly. When exposed to amylolytic enzymes in the human stomach, these pores may represent the site of the first enzyme attack, a way for the enzymes to enter the hilum, or they may be useful for controlling the conversion of starch during germination (Fannon et al. 1992).

Chemistry of Starch–Amylose and Amylopectin

Starch present in granular form in the amyloplasts of tubers and grains and chloroplasts of green leaves have semi-crystalline structure, composed of both amorphous and crystalline rings. The function of both amylose and amylopectin is to store energy in plants and make up the structure of the plant starch granule as a storage unit. There are high- and low-level branching areas in the branched amylopectin molecules. The side chains of amylopectin in the highly branching regions are grouped together to generate the crystalline zones. Thus, short branching chains of amylopectin are the predominant crystalline component in granular starch, while amylose and the branching points of amylopectin add up to the amorphous portions.

The X-ray diffraction pattern of starch depends upon the water content of the granules during the evaluation. Therefore, water can be termed as one of the components that affects the crystalline organization of starch. However, the crystalline to amorphous transition occurs at 60–70 °C in water and the process is coined “gelatinization” (Dufresne 2014). Starch is categorized based upon the X-ray diffraction pattern produced due to the variation in amylopectin chain length and nutritional characteristics as given below (Bashir and Aggarwal 2019).

- (a) Type A: Having amylopectin of chain length 23–29 glucose units and possessing densely packed crystallites with monoclinic lattice, e.g., cereal starch.
- (b) Type B: Having amylopectin of chain length 30–44 glucose units with hexagonal lattice, e.g., potato starches, banana starch, some tropical tubers, and amylose-rich starches.
- (c) Type C: Intermediate between A and B or mixture of type A and B, comprising amylopectin with chain length of 26–29 glucose molecules, e.g., legume starch.
- (d) Type V: Present in swollen granules and is formed upon gelatinization. It is a characteristic of amylose, fatty acids, and mono-glycerides (Gallant et al. 1997).

It is attributed that higher proportions of short and long side chains of amylopectin have a higher crystalline nature. In other words, higher content of longer

amylopectin chains is more crystalline than those with lower content of the long-chain fractions (Singh et al. 2006). The branched amylopectin molecule contains regions with low and high degree of branching. In highly branched regions, side chains of amylopectin are grouped, forming crystalline zone clusters. These crystalline regions are not susceptible to hydrolysis.

The heat of gelatinization (ΔH gel) of such highly crystalline starch is higher than that of high amylose starches. This is due to the increase in double helix content, which increases with higher concentration of long side-chain amylopectin, i.e., the disruption of the double helices rather than long-range disruption of crystallinity increases the ΔH gel. In addition, the higher X-ray diffraction intensities and transition temperature are also higher than that of more extended amorphous region due to higher degree of crystallinity, which provides structural stability and makes the granules more resistant to gelatinization (Singh et al. 2008a).

Amylose varies with a degree of polymerization (DP) in the range of 50–6000 glucose residues. It is now well recognized that a fraction of the amylose molecules is slightly branched by α -(1,6)-linkages (Fig. 2). On the other hand, amylopectin is a very large, highly branched chain molecule with a DP ranging from 30,000 to 300,000 glucose units. Thus, the molecular weight of amylopectin is about 100 times larger than that of amylose. A strand of amylose and amylopectin are respectively reported to have molecular weights in the range of 104–106 and 107–108 Daltons, respectively.

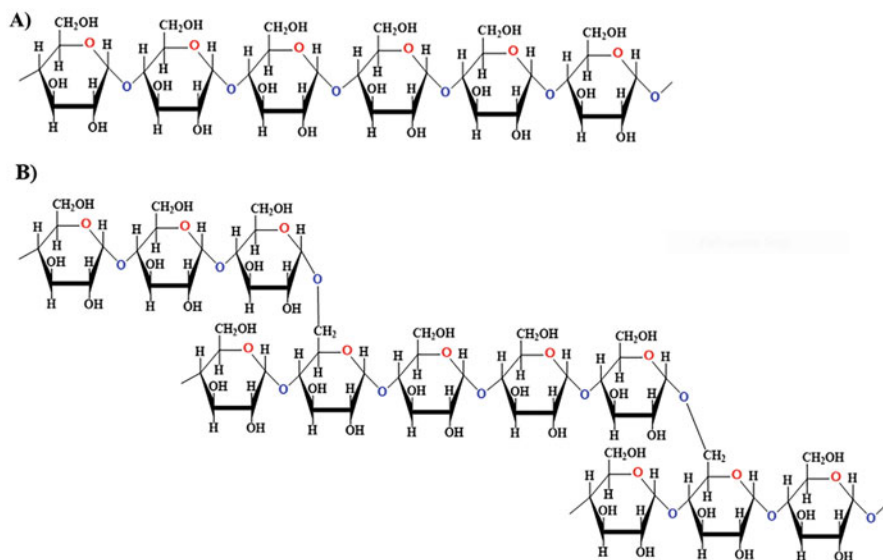


Fig. 2 (a) An amylose molecule made up of a few occasional branch chains and linear, long chains of glucose monomers interconnected by α -(1,4)-glycosidic linkages. (b) A highly branched amylopectin molecule having linear α -(1,4)-glycosidic linkages and branches connected by α -(1,6)-glycosidic bonds

Although amylopectin molecules are shorter than amylose molecules in length, they are more hefty than amylose because of their extremely branched structure. Amylopectin's side chains are A, B, and C, with the C chain serving as the principal chain to which the B branches are connected. The B chain participates in several clusters and is categorized as B1, B2, B3, etc., according to their participation in the side-chain clusters. The subscript indicates how many chains are involved in the development of the cluster. Branched molecules have a chain that creates a α -(1–6) bond to B1 chains at their exterior (Robin J et al. 1974). The features of high amylose starches have been thoroughly investigated. Wu et al. (2006) investigated the ethanol fermentation efficiency of several starch sources and discovered that starches with more than 30% amylose have a negative impact on the efficiency of ethanol conversion. As the amylose content grew, the conversion efficiency dropped. Starch must be gelatinized since it cannot be digested by enzymes in its natural state. This causes it to change into an amorphous mass and make itself more accessible to the glycosidic enzymes.

Native Versus Modified Starch

Starch can be categorized into three major groups: native starch (which is not subjected to any treatment), modified starch (in which few original properties of the native starch has been changed or modified by enzymatic, chemical or physical treatment), and hydrolyzed starch (which causes the polymeric chains to break down into simple sugars like maltose) (Chung and Liu 2009). The primary goal of starch modification is to change the physicochemical properties of native starch in order to enhance functional properties such as gelling, adhesiveness, binding, thickening, and film formation. Because of superior functional qualities than native starches, modified starches are employed in a wide range of processed foods. Many physical modification methods, such as heat moisture treatment (HMT) and annealing, lead to physical alteration of starches without gelatinization or degradation to the starch granules, and have no effect on the granule's birefringence, sizes, or shapes. Treatments affect the interior granule structure in quite distinct ways (Chung and Liu 2009).

Native starch is the pure form of starch occurring in nature, obtained from wheat, corn, rice, potato, tapioca, etc. The components of starch contribute to its water-holding capacity, and swelling in hot water at different temperatures depends on their genotypic diversity and growing conditions, but they are usually insoluble in cold water.

The stability of native starch under different pH values and temperatures varies adversely. Native starch granule is insoluble in water at room temperature and is extremely resistant to hydrolysis by the amylase enzyme. Due to their limited functionality under ordinary circumstances, they have to be modified in order to enhance their properties and functionalities such as solubility, texture, viscosity, and thermal stability. Such modifications are desired for the product development as well as their potential role in the starch industry. Most native starches are semi-crystalline

with crystallinity of about 20–45% (Zobel 1988). For commercial applications, starch is extracted from plants to get a flour-like tasteless and odorless white powder, insoluble in cold water. This powder, which contains native starch, consists of microscopic granules with diameters depending on the botanic origin, ranging from 2 (for wheat and rice) to 100 μm (for potato), and with a density of around 1.5 g. cm^{-3} . This extracted flour is modified by refining through wet grinding, sieving, and drying methods (Buléon et al. 1998).

Starches require modification since they are indigestible and lack the ability to contribute to the structure of many foods. The modification processes involve chemical, physical, or enzymatic treatments, irradiations, etc. In addition, they are modified to acquire different properties that are desirable for different applications such as preparation of packaging material, thickeners, binders, emulsifiers, food, pharmaceuticals, etc.

Physical modification can be brought about by attrition methods such as milling whereby the smashed or “damaged” starch granules are easily dispersed in cold water to provide pastes or gels, which are digestible. “Pre-gelatinized” starch is obtained by the simultaneous gelatinization and drying of aqueous starch dispersions for commercial applications. Enzyme hydrolysis is employed to obtain starches particularly for food-grade materials such as the production of dextrans and glucose. α -Amylase and amylo-glucosidase are most often used in this respect to make glucose syrup, but maltodextrins and crystalline glucose (dextrose), specialist dextrans and maltose may be obtained with β -amylase action. Chemical and physical properties can be appreciably altered by chemical modification of starch, and the chemical modifications usually enhance desirable characteristics of starches. These include “cross-linking” to provide chemical and thermal resistance and the addition of specific chemical moieties to prevent retrogradation of starch gels on cooling (Tester and Karkalas 2002). Chemical modifications include acid modification of starch granules, oxidation, formation of ester derivative, and etherification. These treatments help to improve hydrophilic character, introduce hydrophobic character, introduce positive or negative charges, cross-link the starch chains, and introduce color to the starch. The properties of modified starch depend on the nature of starch source, the amylose to amylopectin ratio, the nature of the substituents that are added, the degree of substitution, whether it is pre-modified by acid hydrolysis, and whether the starch is granular or gelatinized (Robyt 2008).

Starch chain interactions, gelatinization temperatures, granule swelling, acid and enzyme hydrolysis, viscosity, amylose leaching, and retrogradation were all affected by physical modification methods such as in osmotic pressure-treated starches (Pukkahuta et al. 2007). The cross-linking method alters some of the functional characteristics of starches, allowing for a wide range of uses. Because of significant features such as low gelatinization temperature, high swelling and solubility, storage and good cooking durability, acetylated starches with low degree of substitution (DS) have been widely employed in the food industry for several years (Wang and Wang 2002). Acetylated starches are less susceptible to retrogradation because the amylose fraction, which is predominantly responsible for starch retrogradation, is altered. Acetylation appears in all amorphous regions even in the outer lamella of

crystalline areas, resulting in an increase in the specific surface area of starch granules, which is consistent with Biliaderis' (1982) assumption for acetylation in starch granules. Chemical structures, DS, and acetyl group distributions, all influence the physicochemical characteristics of acetylated starches. The DS improved as the size of the starch granules decreased. Taro starch had the greatest DS level and the smallest starch granule dimension, while potato and corn starch had the opposite dimensions. The DS of isolated amylose populations of various granule fraction sizes remained unaltered.

Heating cassava starch at low temperature (45–65 °C) leads to minor variation in structure of starch and amylose–amylopectin ratio (Wicaksono et al. 2016). The number of freeze–thaw cycles increased when starch is heated to a high temperature (59–79 °C) accompanied by freezing and defrosting. It altered starch rheological properties and increased swelling power, heat stability, and viscosity. It also impacted the starch granules' surface characteristics (Lawal 2019). A high pH causes partial breakdown of cassava starch granules, resulting in a reduction in molar size and gyration radius while low pH causes starch to hydrolyze especially in the amorphous region of granules and also lowers the molecular weight of the starch (Wicaksono et al. 2016). Addition of carboxymethyl substitution of hydroxyl groups in starch leads to the addition of the hydrophobic groups on the starch molecule. This increased the starch stability in aqueous media, decreased the ability of recrystallizing, and also prevents the damage due to heat and microorganisms (Lawal 2019). Amini et al. (2015) reported that the granular crystallinity and morphology of surface of the corn starch improves when microwave radiation is applied at various moisture and temperature ranges. This also improved the solubility, emulsifying activity, gelling ability, water and oil-holding capacity, and swelling ability. Pasting temperature and viscosity were found to be increased while the degree of relative crystallinity, gelatinization, and peak viscosity decreased. Another study on modified corn starch by Pukkahuta et al. (2008) showed that the starch granules' shape was distorted, crystallinity was changed from B-type to A-type, and gelatinization temperature was increased when corn starch was heated in a sodium sulfate hypertonic solution at 100–120 °C. Berski et al. (2011) showed that amylopectin depolymerization occurs due to increase in linear molecules when oat starch was chemically reacted with sodium hypochlorite. Rice starch when treated with ultrasonic waves resulted in distorted starch granules, reduced pasting ability and starch digestibility, whereas the viscosity, granules' swelling capacity, and solubility were increased after the treatment (Yu et al. 2013). Thirumdas et al. (2017) treated rice starch with glow discharge or low-temperature plasma leads to free radical generated starch cross-linking and increased leaching of amylose. It increased the viscosity and pasting and decreased the tendency of retrogradation. The same study also showed that the reaction between the acetyl group and hydroxyl group of polymeric rice starch prevents the starch granules from crystallizing and retrograding and also decreased the solubility and pasting temperature. The treatment leads to an increase in swelling capacity and viscosity of starch granules. Another study revealed that there was alteration in the starch reactivity and its physical properties when starch was co-polymerized with synthetic polymers like polyvinyl chloride,

polyethylene, and polypropylene, which change the starch structure from homo to heteropolymer (Hernández 2018). Rice starch when treated with pullulanase (debranching enzyme) resulted in the formation of linear short-chain molecules that were easier to align and aggregate and associate to form double helix. The gelatinization temperature was found to be more in comparison to native rice starch and crystalline structure was also changed from A-type to V-type. Treatments also increased the resistant starch content and water-holding capacity of rice starch (Cao et al. 2020).

Application of ionizing radiation such as gamma radiations to starch also improves their properties such as viscoelastic properties, emulsion stability, etc. Usually application of gamma radiation to polysaccharides results in their degradation, but in the presence of an alkyne such as acetylene enables their cross-linking, leading to the formation of products with enhanced functionality (Al-Assaf et al. 2007).

Amylose to Amylopectin Ratio of Native Starch Species

The ratio of amylose to amylopectin and the structural variations are highly influenced by the source of the plant material. Waxy starches contain less than 10% amylose, whereas high-amylose starches contain amylose greater than 40%. Regular starches consist of 20–30% amylose and 70–80% amylopectin (Tester et al. 2004). Table 1 lists several common native starch species along with the % composition of amylose and amylopectin.

The amount of amylose and amylopectin in the starches of different species and the same species developing under various conditions varies greatly. When scientists examined the starch content of various cultivars of a single species, they discovered clear differences in the species' characteristics. Native starches, picked from suitable cultivars with special qualities, have been proposed as a possible replacement for chemically altered starches. Singh et al. (2008b) investigated the structural, thermal, and viscoelastic characteristics of potato starches isolated through selective breeding of various plant varieties. Additionally, there were significant differences in the distribution of amylopectin chains across the starches, with polymerization levels ranging from 6 to 30. They discovered that changes in genotype and environmental factors had no effect on the amylose content. Thus, the crystalline and amorphous areas of the starch granules are significantly impacted by this variation in the amylose to amylopectin ratio. According to research by Barichello et al. (1990), starches with higher levels of crystallinity had higher transition temperatures and gelatinization enthalpies than those with higher levels of amorphity (Singh et al. 2003).

The amylose/amylopectin ratio's impact on glass transition temperature (T_g) was examined by Liu et al. (2010) since it is relevant to both science and industry. In their experimental work, they used corn starches with various amylose/amylopectin ratios. T_g was discovered to increase from 52 °C to 60 °C with an increase in amylose concentration from 0 to 80 for samples with roughly 13% moisture. T_g rises

Table 1 Percentage composition of amylose and amylopectin and the corresponding ratio in some common native starch species

S. no.	Native starch species	Amylose (%)	Amylopectin (%)	Ratio (amylose/ amylopectin)	References
1.	Normal corn starch	30	70	0.43	Escobar-Puentes et al. (2020)
2.	Agro (corn starch)	28.7	71.3	0.40	Escobar-Puentes et al. (2020)
3.	Great value (corn starch)	27.6	72.4	0.38	Escobar-Puentes et al. (2020)
4.	Brazilian Maizena Dureya (corn starch)	27.8	72.2	0.39	Escobar-Puentes et al. (2020)
5.	Yoki (corn starch)	27.4	72.6	0.38	Escobar-Puentes et al. (2020)
6.	Sweet polvilho	23.8	76.2	0.31	Escobar-Puentes et al. (2020)
7.	Japonica rice	15.4	84.6	0.19	Nishi et al. (2001)
8.	Indica rice	25	75	0.33	Zhou et al. (2016)
9.	Wheat	22.9	77.1	0.31	Slade et al. (2012)
10.	Barley	29.9	70.1	0.43	Regina et al. (2010)
11.	Potato	18	82	0.22	Martens et al. (2018)
12.	Pea waxy	0	100	0	Martens et al. (2018)
13.	Pea regular	27	73	0.34	Martens et al. (2018)
14.	Barnyard millet	33.33	66.67	0.50	Verma et al. (2018)
15.	Finger millet	37.6	64.9	0.54	Verma et al. (2018)
16.	Foxtail millet	31.33	68.67	0.46	Verma et al. (2018)
17.	YNP7 (barley)	15.27	43.54	0.35	Fan et al. (2019)
18.	YNP11 (barley)	16.11	46.50	0.35	Fan et al. (2019)
19.	HCM (barley)	17.63	48.01	0.37	Fan et al. (2019)
20.	EM507 (barley)	14.88	42.98	0.35	Fan et al. (2019)

for the same type of starch as the amylose level does. As a result, T_g rises along with the amylose/amylopectin ratio value.

Effect on the Morphology of Starch Granules

The characteristics of starch related to the morphological structure primarily resolve the significance and applications of starch. The biochemical mechanisms of starch accumulation and the grade and quality of the raw material play a major role in determining the characteristics of starch shape. The genotype of the plant and the

metabolic characteristics of the chloroplast or amyloplast determine the form of the starch granules. The size of the granules varies depending on the temperature of growth. Greater granule size and lower pasting and transition temperatures are created in starch tubers grown in lower-temperature regions compared to those grown in higher-temperature locations. The fluctuation in binding moisture, amylose, and phosphorus content at higher growth temperatures is to blame for this (Tester et al. 1999). Smaller starch granules swell worse and require a greater temperature to gelatinize, in addition to being more difficult to store and tasting worse than larger ones (Zabolotets et al. 2019). It has been noted via scanning electron microscopy (SEM) micrographs that some starch granules, such as those made of rice, corn, wheat, etc., have less smooth surfaces than those made of potato, which have very smooth surfaces. These uneven and smooth surfaces could have tiny pores (Martens et al. 2018).

From an evolutionary standpoint, the creation of various biochemical and physiochemical features of the starch molecule is directly influenced by granule form. The morphological variance among the various starch molecules was primarily controlled by the ratio of two -glucan chains (amylose and amylopectin), as well as their molecular architecture. According to reports, there are numerous factors that contribute to the differentiation and morphological variation of starch granules, including numerous, complex genetic controlling factors, plastid size and number, environmental factors at the time of seed germination, intricate biochemical cascades, and the availability of malto-oligosaccharides (Shapter et al. 2008). According to earlier research, the botanical origin from which starch is obtained has a significant impact on the starch granule's form. Starch granules are created in the plastids known as amyloplasts during the formation of the endosperm. Because of the existence of the surrounding granules, the formation of numerous granules in a single amyloplast causes smaller granules to take on a polyhedral structure. Low magnification reveals that these polyhedral starch granules eventually compacted and appeared as a single granule in the amyloplast. On the other hand, the individual amyloplasts' starch granules of the *Panicoideae* family form a big, simple granular structure. According to Rahman et al. (2000), *Triticeae* exhibit a distinctive bimodal structure in the endosperm where giant lenticellular A-type granules (16–26 μm) interact with small spherical or ovoidal granules (2–10 μm in diameter). The distinctiveness of each starch molecule is typically primarily observed through granular appearance. For instance, compared to legume starches, cereal starches have a lower amylose percentage and smaller granules. Rice starch granules are very small (3–10 μm), have a unimodal size distribution, and are polygonal or angular in shape, but legume starch granules range in size (4–80 μm) and can be oval, spherical, elliptical, or irregular depending on the source plant (Acevedo et al. 2022).

According to evidence on the effects of different amylose/amylopectin ratios on starch morphology, acylation is one chemical modification that stimulates morphological changes, which in turn introduces different functional groups into the starch granule and alters the physical and biochemical properties of starches (Zhu et al. 2017). Wang et al. (2018) emphasized the contribution of starch branching enzyme I (SBEI) and SBEII, one of the genetic regulating variables, to the formation of

morphologically distinct (heterogeneous) starches in some mutant rice. According to this study, gradually lowering the levels of SBEI, SBEIIa, and SBEIIb in transgenic resistant starch rice line (TRS) primarily increases amylose formation and decreases amylopectin content by creating more room for amylose biosynthesis and decreasing amylopectin synthesis or branching degree. Additionally, Verma et al. (2018) used SEM and FTIR to investigate the morphological characteristics of various cereal starches with variable amylose/amylopectin ratios and ended up finding that Basmati and IRRI rice varieties have polygonal, irregular shapes with shiny surfaces, which largely depends on the biochemistry of the chloroplast or amyloplast, as well as plant physiological conditions. According to a report by Waleed et al. (2021), fermentation directly affects the molecular and morphological characteristics of barley starch from the Chinese highlands. The starch granules of fermented barley starch FHB72 and FHB42 were reported to have damaged and cracked structural features without any growth rings, which may be the result of superficial erosion of the starch granules. According to Han et al. (2020), starch granules are made up of amylopectin fragments with large molecular masses in the outer section and a gel-like structure with empty spaces in the inside part. As a result, starch granules produced as a result of high hydrostatic processing pressure (HHP) have severely distorted interior structural morphology. In a similar manner, Liu et al. (2016a) demonstrated that the morphology of the starch granules in sorghum remained unaltered with HHP of 120–360 MPa, but when 480 MPa pressure was applied, the starch granules swelled and collapsed to demonstrate deformation and fusion following application of 600 MPa HHP. These results were also supported by Liu et al. (2016b) in mung bean starch, Deng et al. (2014) in rice starch, Liu et al. (2012) in corn starch, Liu et al. (2008) in potato starch, and Liu et al. (2008) in rice starch. These results made it abundantly clear that the amylose/amylopectin ratio and degree of branching have a significant impact on the granular morphology. Another study by Dhull et al., published in 2021, examined the effects of physical and chemical alterations on the morphological characteristics of the starch in black rice (*Oryza sativa* L. *indica*). They discovered that native rice starch had smaller irregular forms with sharp angles, edges, and no pores on the surface before heat moisture treatment (HMT).

HMT, however, has a considerable impact on the shape of rice starch granules, and starch fragment aggregation has also been noted. Similarly, the surface of starch granules modulated by octenyl succinate anhydride (OSA) was observed to be rough and dotted with pores. The primary way the OSA functional group affects surfaces is by generating many holes and channels. Al-Ansi et al. (2022) also suggested that as germination progressed, amylose percentage increased from 22.81% to 26.63%, while starch content decreased from 45.70% to 31.67% (24 h, 48 h, 72 h). The increased amylose/amylopectin ratio during germination supported the theory that the grains' starch had been mobilized. Amylase was more accessible to the non-reducing ends of amylopectin than to those of amylose, which contributed to the greater amylose/amylopectin ratio. These findings have consequently drawn attention to the fact that the morphological characteristics of starch granules have the potential to enhance the quality of starch-based meals and other uses.

Effect on the Structure of Starch Crystallinity

As in section “[Granule Structure of Starch](#),” starches are divided into three crystalline forms known as A, B, and C depending on the XRD results. The assembly of amylopectin double helices into crystalline lamellae, interspersed with amorphous lamellae made up of -(1–6) branch regions of amylopectin and amylose in waxy starches, is what causes crystallinity in starch granules. It is evident that the alternating crystalline-amorphous lamellae serve as the foundation for semi-crystalline (or “dark”) growth rings, which consist of about 16 crystalline lamellae and are roughly the same width as the interspersed amorphous (or “light”) growth rings (–140 nm), in high-amylose starches, where amylose forms double helices and crystalline domains accordingly (Tester et al. 2004). While nanocrystals made from B-type starches created round-like particles, those made from A-type starches adopted square-like particles. The various packing arrangements of the amylopectin chains for A- and B-type starches provided an explanation for this. Starch produces a compact semi-crystalline shape in order to increase the concentration of energy stored and decrease the amount of storage energy used. Additionally, a number of integrated enzyme systems are used in this biochemical process.

According to a variety of terminologies, such as starch molecular fine structure, structures formed by amylose–amylopectin fine structure, and starch aggregate structure, structures formed by amorphous lamellae, crystalline lamellae, fractal structures, and ordered aggregates, have all been thoroughly investigated (Chi et al. 2018). Therefore, a fundamental understanding of the starch hierarchical changes is important in order to evaluate the impact of starch crystallinity on the food processing industry as well as other applications. Using chain length distributions (CLDs) and full molecular size distributions, Tao et al. (2019) emphasized the molecular fine structure of starch from this perspective. Additionally, they used a variety of technologies to reveal the hierarchical branches of CLDs, such as size-exclusion chromatography (SEC) or its coupling with multi-angle laser scattering (SEC-MALS), asymmetrical flow field-flow fractionation coupled with MALS (AF4-MALS), fluorophore-assisted capillary electrophoresis (FACE), and high-performance anion-exchange chromatography (HPAEC). Molecular fine structure is primarily framed using a biosynthesis-based model, highlighting changes throughout starch biosynthesis, according to a different study by Nada et al. (2017). Additionally, using various technologies like X-ray diffraction (XRD), SEM, atomic force microscope (AFM), confocal laser scanning microscope (CLSM), transmission electron microscope (TEM), primarily by small-angle neutron scattering (SANS), and small angle X-ray scattering (SAXS), starch aggregate structures of 1–100 nm size are interlinked with lamellar structure, fractal structure, as well as nanoscale reassemble (Blazek and Gilbert 2011). While AFM/SEM/TEM focuses on specific positions of starch nanoparticles, XRD unravels crystalline and amorphous structures on a small scale. Starch structures that are hundreds or thousands of nanometers in size are also interpreted using SAXS and SANS. As a result, a thorough analysis of each approach gradually discloses all of the structural characteristics of starch crystallinity. Additionally, the fundamental -D-glucan chains of starch ultimately control structural organization (Wang et al. 2019).

Previous research (Shi and Seib 1992) identified a number of starch self-assemble structures with reduced DP and amylopectin chain length. First, DP 10 chains were predicted to form double helical structures far less frequently than chains with DP 14–24 or DP 12–22. Additionally, double helices are once more organized in order to create native crystalline structures of the A-type, B-type, and C-type, while another V-type crystallinity is created after being processed with a variety of guest molecules (lipids, glycerol, DMSO, etc.) (Krishnan et al. 2020). However, according to a few studies (Bertoft 2017), while tuber starches have B-type crystalline structure because of their long-chain amylopectin chain length ($DP > 20$), cereal starches have very short amylopectin chain length (DP20), which results in the formation of A-type crystalline structure. The simultaneous existence of both A- and B-type crystalline structures is represented by the C-type crystalline structure, which is primarily found in legumes, roots, fruits, and other stem starches. Furthermore, it is confirmed in a study by Rodriguez-Garcia et al. (2021) that while B-type crystals have hexagonal structures, which lead to more open structures and packing with 36 water molecules within crystallinity, A-type crystals have polymorphic crystals that are packed with eight water molecules, resulting in orthorhombic structures. Since amylose–amylopectin arrangements in the starch sources are finally deciphered by the type of crystallinity and thorough indexing of orthorhombic as well as hexagonal structures, it is important to understand these factors. According to Jane et al. (1997), A-type starches generate thinner crystalline lamellae than B-type starches because they have very short amylopectin chains (Chi et al. 2017). Due to the inclusion of “weak places” into the structure of crystalline lamellae, it is also extremely vulnerable to acid and enzymatic treatments. This suggests that starch hierarchical structures are thus intrinsically linked and driven by starch intermolecular interactions with various characteristics. This principle of starch multiscale structures also has a direct influence on starch digestibility, targeted food preparation, and in other aspects.

Effect on Biochemical Properties

The solubility, gelatinization, retrogradation, glass transition, hydrolysis, oxidation, esterification, digestibility, and reinforcing qualities of starch are just a few of its physical and biological characteristics. The amylose to amylopectin ratio, molecular weight, and extent of branching, among other factors, have a significant influence in predicting the functions of a specific starch sample, including pasting, texture, rheological properties, digestibility, etc. (Egharevba 2019). By removing flaws in its natural qualities, various adjustments to these starch traits improve their functional applications.

Digestibility

Starch crystals are less prone to being digested because they contain bonded liquid. This explains why A-type starch dissolves more quickly than B- and C-type starch

crystals. Smaller granules digest more quickly than larger ones because they have more surface area to interact with digestive enzymes (Franco et al. 1992). Since amylopectin is easier to digest and amylopectin presence slows down the rate of starch digestion, amylose is known to contribute more to resistant starch (RS) or slow-digesting starch (SDS). Therefore, greater crystallinity is linked to easier digestion, while amorphous fraction decreases digestibility. High amylose content in starch is resistant to digestion, slowing the rate at which blood sugar levels rise. As a result, it has a low glycemic index and is less likely to gelatinize (Magallanes-Cruz et al. 2017). Consequently, the glycemic index lowers when the amylose to amylopectin ratio rises. This suggests that amylopectin is readily digested and hydrolyzed, which is harmful to diabetics since it releases reducing sugars into the bloodstream (Dipnaik and Kokare 2017). Higher amylopectin content starches break down more quickly, earning the moniker “rapidly digested starch” (RDS).

Digestion and cooking are positively correlated with each other. Cooking, presumably by gelatinizing it, increases the starch’s availability for enzymatic hydrolysis (Snow and O’Dea 1981). The digesting process is also impacted by the starch granules’ surface shape. The surface of starch granules might be smooth like rice or squishy like a potato, and the amount of holes is thought to affect how well starch is digested. As a result of these channels, it was thought that enzymes would break down starch granules from the inside out, resulting in a quicker digestion. However, Martens et al. (2018) found that the positive correlation between the number of pores and the digestion kinetics could only be applied within a single botanical source and not across botanical sources.

High amylose starches have been found to have high gelatinization temperatures and to produce tougher gels (Shi et al. 1998). In the presence of water, a gelatinization process occurs that facilitates digestion (Tester et al. 2000). The granules of native starches undergo a characteristic structural reformation that is unique to their botanical origin when heated in excess water. They inflate and gradually lose their ordered or crystalline structure, turning into a basically amorphous mass. Eventually, a thin paste or gel is created and the granule structure is completely gone. Due to the heating of the extra water, the double helices at this point are hydrated and begin to unravel. It is believed that this process starts at about 45 °C (onset temperature, or T_o), peaks at 60 °C (T_p), and is finished about 75 °C (T_c). Amylases and amyloglucosidase, starch-hydrolyzing enzymes, eventually cause starch to be totally digestible. However, starch is fermented in the human colon and produces short-chain fatty acids, which are subsequently absorbed and used as a source of energy.

Dispersibility

Reconstitution of flour or flour mixtures in water is known as dispersibility. It has been said previously that the better the dispersibility, the better the flour will reconstitute in water (Kulkarni et al. 1991). To put it another way, it is the starch’s capacity to swiftly form tiny granules when mixed with water in the spray tank to produce a fine particle suspension. For products made from starch, starch

dispersibility in organic solvents is essential because it impacts the production procedures, which in turn affects the final product's attributes. The finished product is stable, consistent, and has high physicochemical qualities thanks to a well-dispersed powder, which supports its use as a thickening, stabilizing, and binding agent in many dishes, such as soups, sauces, gravies, baked goods, etc. Particle sizes, z-potential, moisture, and other variables can all affect how well starch granules dissolve in organic solvents. In their 2009 study, Akanbi et al. compared the dispersibility of breadfruit and cocoyam starches and discovered that cocoyam starch had a higher dispersibility value (86.500.18%) than would breadfruit starch (40.6670.5774%).

The Kulkarni et al. (1991) approach, as updated by Akanbi et al. (2009), can be used to measure the dispersibility of starch. A 10 g sample of starch is precisely weighed into a 100 ml measuring cylinder with a stopper. Also, 100 cc of distilled water should be added, stirred thoroughly, and then left to stand for 3–4 hours to develop the pure and sediment layers. The dispersibility is determined by subtracting the upper layer meniscus from the lower layer meniscus or by subtracting the volume of settled particles from the final volume (Jacob and Adeleke 2019). Both biomaterials and biological applications depend on dispersibility, with monodispersed materials frequently being the most desirable (2013) (Ha, S., et al.).

Che et al. investigated the dispersibility of Cassava starch particles of various sizes in liquid paraffin (2007). They claimed that compared to the native cassava starch, the micronized cassava starch dissolved more easily in liquid paraffin. This demonstrated how accurately reducing granularity increased dispersibility. Native cassava starch's large granule size resulted in decreased dispersibility. The particles' intrinsic gravity caused them to settle swiftly. During starch suspension heating, the enormous variation in granule size and swelling power amongst blended starches results in an uneven distribution of moisture. As a result, the behavior of the combination deviates from what may be predicted based on the behavior of the particular starches. There were significant variations ($P < 0.05$) in the values of dispersibility for the control starches and their respective blends, according to Ashogbon's (2014) research on bambarra groundnut (BBS) and cassava (CS) starches and their blends. As was already indicated, the reconstitution of starch flour will work better the higher the dispersibility value. In this investigation, the 30BBS/70CS mixture's value of dispersibility was significantly higher than that of the other starches, and the results are consistent with the dispersibility values published by Ashogbon and Akintayo for rice starch (87.01%) (2013). Additionally, all of the dispersibility values found in this study were superior to those found in Akanbi et al.'s (2009) analysis of breadfruit starch (40.67%).

For their long-term stability and ease of preparation, starches are modified and dried or pre-gelatinized. Compared to untreated starch, which forms gel at room temperature and is more prone to deposition, it absorbs more water and disperses easily in water. When starches are handled in an alkaline environment, cationic starches are created, and they have a higher dispersion than untreated starches (Omorieghe Egharevba 2020). Ren et al. (2018) investigated the chemical, wettability, thermal, and structural characteristics of cross-linked starch nanocrystals (SNCs),

which were modified by cross-linking modification using citric acid in aqueous medium. They discovered that cross-linked SNCs had lower polarity, stronger hydrophobicity, and dispersion stability, which allowed them to be used as reinforcements in hydrophobic polymer matrices.

Rheological Properties

Because they can change the technical and mechanical qualities of the starch, starch rheological properties are extremely important for the quality of products associated to starch (Zhang et al. 2017). Amylose/amylopectin ratio, particle size, and amylose-lipid composition and interactions, particularly lipids and proteins that prevent the interaction of starch and heat, have an impact on pasting profiles (Liu et al. 2003). Two corn flours, Kassai, which is white, and Atp-Y, which is yellow and has a different amylose/amylopectin ratio, were examined by Dongmo et al. (2020) under unroasted, roasted, and unfermented conditions. The tested flours' pasting temperatures ranged between 74.50 and 77.77 °C and were influenced by the variety and treatment; the yellow variety demonstrated a higher pasting temperature value than the white variety. This is because the yellow variety has a large concentration of lipids, which interact strongly with the amyloidosis and reduce its swelling. Similar to this, the untreated flours had higher pasting temperatures than the treated ones due to the significant lipid and protein content, as well as the strong connectivity between these components that serve as a temperature barrier. The decreasing concentration of lipid and protein following the treatment and the beginning of hydrolysis of the minimal energy interactions that maintain the crystalline structure of the starch are what cause the drop in pasting temperature in various treatments. The peak viscosity value of the untreated and unfermented flours was greater. The stronger hydrolysis of the starch constituents during heat treatment, loss of phosphorus ions, and reduction in interactions that maintain the starch's crystalline structure could all be contributing factors to the lower peak viscosity values (431.50 Cp and 895.50 cP for Atp-Y and Kassai, respectively) in roasted flours. The setback value varied from 356 to 3198 cP with a decline following the various treatments, which may be caused by the untreated maize flours' high amylose/amylopectin ratio and the yellow variety's high lipid content (Mishra and Rai 2006). According to Casthana et al. (Castanha et al. 2021), the peak apparent viscosity of several starches with differing amylose contents was ordered as follows: potato (amylose 28%), cassava (amylose 20%), waxy maize (amylose 2%), maize (amylose 28%), and high amylose maize (amylose 72%). This behavior was greatly influenced by the inherent characteristics of starch, granule size and shape, amylose/amylopectin ratio, and their individual chain lengths (Tester and Morrison 1990). Amylose content of maize starch is adversely connected with peak viscosity, final viscosity, trough viscosity, setback value, and breakdown viscosity, according to Karakelle et al.'s (2020) study of the rheological properties of the maize starches that varied in their amylose content. The pasting temperature was seen to decrease along with the reduction in amylose level (from 70% to 0%). The samples with a 20% or lower amylose content, however, did not

significantly differ in pasting temperatures. According to the kinetic modeling of pasting curves, all starches had starch coefficients greater than 1, indicating that water absorption into the starch granules increased the rate at which the granules swelled. In comparison to normal maize and rice starches [82.0 and 79.9 °C, 152 and 113 rapid viscosity unit (RVU), respectively], waxy maize and waxy rice starches had lower pasting temperatures (69.5 and 64.1 °C, respectively) and higher peak viscosities [205 and 205 rapid viscosity unit (RVU)], respectively. The primary component of starch, amylopectin, is really responsible for the ability of starch to expand and the development of viscosity during cooking, whereas amylose, particularly in the presence of lipids, appears to interact with amylopectin and limit starch granule swelling. As a result, normal maize and rice starches displayed higher setback values (74 and 64 RVU, respectively) than their waxy counterparts (16 and 16 RVU, respectively) (Ai and Jane 2015). Due to the depolymerization of starch molecules, acid-thinned sorghum starch had less viscosity (Singh et al. 2009). Due to the starch molecules' depolymerization and charge repulsion, oxidized cassava and barley starches have poorer gel strength than the corresponding control starches (Sangseethong et al. 2010). In comparison to their control starch, annealed mung bean starch (Chung et al. 2000), HMT rice (Hormdok and Noomhorm 2007), or potato (Eerlingen et al. 1997) starch typically forms a stronger gel. The rising effect also appears to be more pronounced for native starches with high swelling capacities, such as potato starch. HMT, however, has been demonstrated to prevent the gelation of sweet potato and rice starch. According to Pimpa et al. (2007), sago starch treated with 10/15 kGy electron beam radiation results in a stiffer gel than their control starch, but when treated with 20–30 kGy dosage, the gel becomes softer. This demonstrated how radiation therapy has an effect on the cross-linking and depolymerization of starch granules (Bao et al. 2005). When Hussain (2020) compared the rheological characteristics of chickpea and rice starch, he discovered that rice starch had lower values for the corresponding final, peak, and setback viscosities. All starches displayed shear-thinning non-Newtonian behavior when the parameters of steady shear were investigated (n1). Another study on modified potato starch revealed that when acetylated composite potato starches were compared to their unmodified counterparts, the majority of the pasting properties, such as breakdown, setback, peak, and final viscosities, were found to be decreased (Bolade and Oni 2015). The rheological characteristics of potato starch in their natural, HMT, microwave-treated, ultrasonicated, and synergistic forms were examined by Nadir et al. (2015). When native potato starch was treated with HMT or the microwave, the initial pasting temperature increased (from 61 to 63 and 65 °C, respectively). The initial pasting temperatures were reduced from 56 and 55.2 °C, respectively, with the other two treatments (ultrasonication and syneresis). In comparison to the original potato starch, the setback value of modified starches was found to be lower for all treatments with the exception of the microwave treatment. In comparison to the native approach, the breakdown values of the HMT method were higher. Starches are highly stable during continuous heating and agitation, as evidenced by the drop in breakdown value caused by HMT.

The rheological characteristics of starches with various amylose to amylopectin ratios (80/20, 50/50, 23/77, and 0/100) were investigated by Xei et al. (2009) using samples that had first been pre-compound with water. Under a slit capillary die, the shear stress and apparent melt viscosity properties of sample pellets were studied as functions of moisture content (19–27%) and extrusion temperature (110–140 °C) at various shear rates. Six points were typically recorded for each sample using the cutting and manual entering measurement mode, with the screw speed varying between 30 and 180 points per minute.

Using the equation, apparent shear rates are determined:

$$\dot{\gamma} = \frac{6Q}{WH^2}$$

where $\dot{\gamma}$ is the shear rate, Q is the volumetric flow rate (in cm³/s), W is the slit width, and H is the slit height.

The values of shear stress are determined by the following equation:

$$\tau = \frac{H \cdot \Delta P}{2L}$$

Here, τ is the shear stress, ΔP is the pressure drop over the capillary, and L is the slit length. In the work, the rheological properties of various corn starches with different amylose/amylopectin ratios are studied using a Haake rheometer.

The melts demonstrated shear thinning behavior in all circumstances, and the power law index n ($0 < n < 1$) tended to rise with increasing temperature and moisture content while falling with increasing amylose content. The consistency (K) value increased with increasing amylose while reducing with increasing moisture content and extrusion temperature. Because of their higher levels of gelatinization, increased molecular entanglements between linear polymer chains, and lower levels of gel-balls and super-globes, which are much easier to move than long linear chains, the amylopectin-rich corn starches exhibited lower viscosity and higher Newtonian behavior (Xie et al. 2009).

The energy stored in and recovered from it every cycle is measured by the storage dynamic modulus (G'), and the energy lost or dissipated per cycle of sinusoidal deformation is measured by the loss modulus (G'') (Ferry 1980). Tan can be used to represent the energy storage to energy loss ratio for each cycle. In the dynamic rheometer, the value of G' for starch rises with temperature, reaches a maximum, and then falls as the temperature is raised further. This change results from the granule enlarging and disintegrating due to the melting of the crystalline areas (Evans and Haisman 1979). Thus, the rheological properties are significantly influenced by the granular structure. For example, huge and irregularly shaped potato starch granules displayed higher storage and loss modulus and lesser tan than the smaller, oval-shaped granules (Singh and Singh 2001). Protein content raises the pasting temperature, while damaged starch and protein content lower the peak viscosity value

(Singh et al. 2003). Additionally, the levels of G' , G'' , and \tan decrease with an increase in amylose and increase with an increase in amylopectin (Kaur et al. 2002).

Reinforcing Properties

Because starch is widely available, inexpensive, renewable, biocompatible, biodegradable, and nontoxic, the usage of starch-based materials is drawing a lot of attention. These qualities make them a top candidate for both drug carriers and implant materials. It has also been discovered that starch nanocrystals make good reinforcements. However, the starch's botanical source may have an impact on its ability to reinforce. LeCorre et al. (2012) investigated how the botanical origin of starch nanoparticles affected the starch-natural rubber nanocomposite's reinforcing characteristics. When employed as filler, starch nanoparticles extracted from different types of potato, maize, and wheat improve the material's reinforcing characteristics. With the exception of potato starch, the reinforcing effect diminished as the amylose level rose.

Reinforcing properties are one of the key biological aspects of starch molecules that make them useful for a variety of applications. Recently, Bangar et al. (2022) published a work in which cellulose nanocrystals (CNC) from the Kudzu (*Pueraria montana*) vine were used to reinforce starch-based nanocomposites. In this study, kudzu CNC was used to reinforce pearl millet starch, which enhances crystallinity, heat and moisture-barrier characteristics, and tensile strength following reinforcement. Nanocomposite films were developed utilizing solution-casting procedures. Due to the strong compatibility of intermolecular H-bonding contacts, chemical similarity, enhanced dispersion, and adhesion capabilities of Kudzu CNCs in the PMS matrix, the stability performance of PMS/Kudzu CNC nanocomposites is significantly higher. Another study by Campos et al. (2022) focused on the impact of adding fine bagasse microparticles (FBM) to foamed materials made from cassava starch, which had a substantial impact on the foam's physical and mechanical characteristics following reinforcement. Additionally, it should be emphasized that starch has demonstrated a strong capacity to function as the best reinforcing agent in a variety of contexts recently. Starch-based biodegradable polymers' manufacture, difficulties, and prospects for the future were explained by Siqueira et al. in 2021. They continued by saying that while this way of producing polymers is more involved, time-consuming, and sophisticated than the conventional method using petroleum-based materials; it has significant advantages from both a biodegradability and a business standpoint. Moreover, Lin et al.'s (2011) study demonstrated a reinforcing mechanism using starch nanocrystal filler in polymer matrices to improve intrinsic stiffness, mechanical performance, thermal characteristics, solvent absorption, and barrier properties. It has been discovered that starch nanocrystals' structure is less resilient than cellulose nanocrystals', which restricts their use in the biomaterials sector and would considerably increase their practical utility in the near future. The combination of citric acid, cellulose fibers, and active phenolic extract were utilized as cross-linkers, reinforcement agents, and antioxidant materials,

respectively, in Li's (2020) study on three main techniques of starch films for food packaging. After being strengthened with cellulose fibers, starch's performance was improved in terms of stress resistance and adequate flexibility. It may also be utilized as the ideal coating material to stop the oxidation of lipids in foods like nuts and cereals. According to Liu et al.'s (2020) work on thermoplastic starch film, thermoplastic starch (TPS) was made from cassava starch, glycerol, and nano-SiO₂ using a straightforward film-blowing approach. Tensile strength and thermal stability of starch greatly increased while starch crystallinity simultaneously decreased after the addition of glycerol and nano-SiO₂. Waxy maize starch nanocrystal reinforcement has also been used to describe pea starch films. Tensile strength of the composite films increased with the addition of 5% of nanocrystals, and it was discovered that additions of more than 7% of nanocrystals primarily caused nanocrystals to aggregate on the surface of the composite films and produce longitudinal fibrous structure (Li et al. 2015). According to Lopez-Gil et al. (2014), there are a number of ways to use natural fibers like barley straw and grape trash to enhance the mechanical properties of composites made of thermoplastic starch. Comparatively to oil-based polymers, such as polypropylene (PP), polyethylene-terephthalate (PET), and a biodegradable polymer, Polylactic acid, those starch-based products are appropriate for food packaging trays (PLA). The production of thermoplastic arrowroot starch (TPAS)-based composite films through the insertion of arrowroot fiber (10–15%) into glycerol plasticized matrix utilizing solution casting process was emphasized by recent works by Tarique et al., (2022). After fiber reinforcing, mechanical qualities are improved, and reinforced films show tremendous promise for the food packaging sector. In a similar vein, another study looked at the production of biodegradable plastic using maize starch powder and orange peel powder, which led to an increase in the starch film's mechanical stability and biodegradability. Additionally, it improved the ability of water to absorb moisture and the solubility of water and oil (Chhatariya et al. 2022). In one study, Doan et al. (2019) described how to characterize rice starch gels and how to strengthen them with resistant starch made by enzymatic means. The amylosucrase-modified resistant starch reinforcing procedure resulted in an increase in rice starch gel hardness of up to 60%, but a considerable decrease in gel cohesiveness, which was found to be consistent with the reinforcement. Haaj et al. (2016) compared the reinforcing properties of amorphous starch nanoparticles (SNPs) obtained from ultrasound treatment with those of starch nanocrystals extracted from waxy maize starch through an acid hydrolysis and discovered that the former has better reinforcing properties even though the latter is produced using an environmentally friendly or green methodology.

The reinforcing properties of starch nanocrystals in a matrix of unvulcanized natural rubber were investigated by Mele et al. in 2011. Nonlinear dynamic mechanical experiments were used in their investigation to emphasize the Mullins and Payne effects as well as the starch nanocrystals' substantial reinforcing effect. Two models – the Maier and Goritz model and the Kraus model – were used to forecast the Payne effect. The Maier and Goritz model was used to show that the nonlinear viscoelastic characteristics were controlled by the adsorption and desorption of natural rubber

chains on the filler surface. However, the Kraus model showed that for filler amounts of more than 6.7 volume %, a percolating network formed. Overall, it was noted that adding reinforcement with other materials to the starch components led to both increased functionality and texture. Therefore, the future development of new technologies in the food industries was influenced by the qualities of starch that reinforce other components.

Conclusions and Future Perspective

It has been demonstrated that the crystallinity and granule structure of starch influence both its chemical and physical properties. Both of these depend on the amylose to amylopectin ratio in the starch sample, which in turn depends on the type of plant used and the environment in which it was grown. As a result, the variability in the configuration of amylose and amylopectin as well as their branching distribution within a starch granule accounts for the majority of the variation in the characteristics of starch samples from various sources. The values of various parameters, including Tg, pasting temperature, gelatinization temperature, etc., to name a few, increased as the amylose concentration increased. Therefore, it is ideal to either manage the plant's development conditions or adjust the starch sample to meet a certain requirement in order to obtain starch for a particular use.

Starches are increasingly gaining more attention as a raw material in the production of biodegradable polymers as a result of environmental concerns and the scarcity of fossil fuels (Arvanitoyannis 1999). Since starch is a naturally occurring, biodegradable polymer, it is frequently combined with other substances to improve its physical, chemical, mechanical, and functional properties. There are a wide variety of uses for starch nanoparticles, such as a medicine carrier, emulsion stabilizer, fat substitute, and adsorbent for wastewater treatment (Haaj et al. 2016). It is also widely employed in the food, sweetener, glue, paper, textile, and textile sectors. These uses depend on the characteristics of starch, such as viscosity, swelling volume, solubility, and clarity. Because starch dissolves in water, it can be used as a vehicle to transfer solid active chemicals to a target organism (Jacob, and Adeleke 2019).

As a result of developments in chemical and biotechnology, starch has been widely modified for a variety of uses, and even if its physicochemical qualities have changed, they are still simple to handle. Starch will soon prove to be a predecessor to “green plastic” materials. As a flexible, ecologically friendly resource, starch can be used to replace harmful petroleum-based products in fields such as material science, biotechnology, industry, medicine, and engineering.

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Biopolymers: Building Blocks for the Synthesis of Advanced Materials

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Ritika Tripathi, Akshit Malhotra, Ashwini Chauhan, and
Suchitra Rajput Chauhan

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Abstract

Material synthesis procedures of advanced materials such as nanomaterials and superconductors utilize traditional chemistry processes accompanied by high ecological footprint. These synthesis processes involve high energy, utilize toxic solvents and chemical reagents, and demand complex reaction conditions. Due to the growing demand of such advanced materials in several modern-day commercial applications, there is a strong need for developing sustainable reaction systems that involve lesser costs and energy, minimal environmental toxicity, and ease of large-scale manufacturing. In this chapter, we discuss the importance

R. Tripathi · S. R. Chauhan (✉)

Department of Applied Science, School of Engineering and Technology, BML Munjal University,
Gurugram, Haryana, India

e-mail: suchitra.chauhan@bmu.edu.in; chauhan.suchitra@gmail.com

A. Malhotra

Department of Microbiology, Tripura University (A Central University), Agartala, Tripura, India

Invisiobiome, New Delhi, India

A. Chauhan

Department of Microbiology, Tripura University (A Central University), Agartala, Tripura, India

of biopolymers as ecofriendly materials that can emerge as suitable templates for synthesis of superconductors and reducing and stabilizing agents for synthesis of nanoparticles. Besides, we discuss the green chemistry approaches which can further enhance the sustainability of the synthesis procedures of advanced materials catering to the technological advancements of the society.

Keywords

Superconductors · Nanomaterials · Green chemistry · Biopolymers · Environmental sustainability

Introduction

Biopolymers are long repeating chains of the monomers of sugars (polysaccharide-based biopolymers) or proteins (polypeptide-based biopolymers) sourced from animals, plants, or microorganisms.

The polysaccharides-based biopolymers are composed of glucose units covalently bonded together using glycosidic linkages. The process of polymerization of polysaccharides is accompanied by loss of a water molecule for each glycosidic linkage. Polysaccharides based biopolymers are diversely found in nature which may differ in their overall charge and could be polycationic (chitosan), neutral (dextran), and polyanionic (alginate) (Harding et al. 2015). They can also be classified as homogeneous and heterogeneous polysaccharides if the monomer units are the same or different; for example, glycogen is a homogenous polysaccharide chain, and xanthan gum is a heterogeneous polysaccharide chain (Johnson-Green 2002).

Polypeptide-based biopolymers comprise amino acids as monomer units that covalently bind with repeating units using amide linkages accompanied by loss of a water molecule for each amide linkage. Polypeptide chains further form specialized structures (secondary, tertiary, and quaternary) using molecular interactions like hydrophobic and hydrophilic interactions, disulfide and salt bridges, and hydrogen bonding causing polypeptide chain folding to form beta-plated sheets, alpha-helical structures, and even complex three-dimensional quaternary structures (Enna and Bylund 2007). Some examples of polypeptide-based biopolymers are collagen, elastin, silk-like proteins (SLPs), keratin, albumin, and gelatin.

Polymers obtained from biological sources (renewable resources) have gained enormous attention and are industrially produced as an alternative to fossil-fuel based (a non-renewable resource) polymers. Traditional material synthesis offers several limitations in terms of minimizing carbon footprint. The innate biodegradable nature of biopolymers has reduced the cumulative carbon footprint of any chemical reaction system wherever they are employed.

Polymers made from renewable resources minimize environmental pollution as they are biodegradable and reduce carbon footprint and the risk of global warming. Moreover, biopolymers have found a wide range of applications such as fabrication of photovoltaics and photodetectors, manufacturing of biomaterials, for formulating

food coating and packaging materials, cosmetics, tissue engineering, drug delivery, etc. In the field of material science, the production of biopolymers has gained enormous interest among industry and academic researchers owing to their potential to replace polymers made from non-renewable sources. Biopolymers have also increasingly been used in the green synthesis of advanced and emerging materials like superconductors and nanomaterials. Biopolymers are used as templates, reducing agents, and stabilizing/capping agents for synthesis of *advanced materials: superconductors* and *nanomaterials*. Environmentally benign material synthesis processes are extensively being explored due to adverse environmental impact of conventional synthesis methods and growing demand for materials with unique physical properties in several modern-day applications.

Superconducting materials play an important role in several fields such as medical diagnostics using MRI imaging systems, a study of subnuclear particles like taus and neutrinos using particle accelerators, and operation of magnetic levitation high-speed trains. Another class of advanced materials, that is, *nanomaterials* have found their application in wide areas such as catalysis, drug delivery, electronics, oil recovery, antibacterial textiles, paints and medical surfaces, corrosion inhibition, etc. These advanced materials (superconductors and nanomaterials) are typically synthesized using physical and chemical methods like thermal decomposition, chemical reduction, UV irradiation reduction, laser irradiation, ultrasonication, etc. All these methods employ high energy-consuming machines and specially designed devices to achieve material synthesis conditions such as high-temperature calcination and sintering, exposure to ultraviolet light, reduction process assisted by ultrasonic waves, and use of pure inert gas or high-power intense laser beam. The chemical methods for the synthesis of nanoparticles and superconductors present several limitations such as the use of organic solvents and surfactants which are toxic for environmental disposal. For example, hydrazine hydrate and sodium borohydride are not safe for direct disposal in the environment but are extensively used as reducing agents to prepare nanoparticles such as AgNPs. Besides, methods like hydrothermal synthesis demand extreme precision of experimental conditions such as the amount of surfactant, precursor concentration, and solvent volume and influence the structure of nanomaterials due to variation in different batches of experimentations (Zhang et al. 2022).

The ecofriendly synthesis of advanced materials has gained momentum among researchers due to advancements in green chemistry and the rising need towards sustainability by adopting less toxic synthesis procedures. The biopolymer-assisted green synthesis of advanced materials including nanoparticles and superconductors has gained momentum owing to its facile nature, cost effectiveness, and minimum environmental impact. Biopolymer assisted synthesis allows the control of the nanomaterial morphology due to the presence of specific chemical interactions among biopolymer chains and the precursor substances (in the bottom-up nanoparticle synthesis approach). Besides, biopolymer-based synthesis of superconducting material like Lu123 provides homogeneity of precursor materials at atomic level without the requirement of mechanical grinding which is otherwise done for over 150 h in physical method of synthesis. Moreover, the biopolymer (dextran) backbone provides a microstructure that efficiently controls nucleation and crystal growth of superconductors (Dedman et al. 2010).

Synthesis of Superconductors Using Templated Biopolymers

Superconductors are known for carrying tremendously high currents below the transition temperature. It is the capability of high T_C (transition temperature) superconductor to persist and allow extremely high current density (J_C) (Rajput et al. 2006) at relatively high temperature (normally higher than 40 K) that has made these a sought-after material viz., YBCO, BISCO, and MgB_2 (Chauhan and Chaudhary 2010; Rajput 2017). J_C is affected by defects, grain boundaries, impurities, and their size as well (Rajput and Chaudhary 2008). For a given high T_C superconductor, thus enhancing J_C needs attention toward its fabrication processes under various required morphologies (Rajput and Chaudhary 2010). Additionally, most of the technologically important high T_C superconductors are oxides viz., YBCO, BISCCO, etc., and need to be calcined under a flowing oxygen atmosphere. Epitaxial growth or stability of high T_C phase of a superconductor has also been a constant objective of researchers to enhance J_C and H_C (critical magnetic field) (Gogia et al. 1991).

Achieving the Meissner effect (diamagnetism) is challenging in nano dimension owing to the suppression of T_C due to a decrease in electron density of states (DOS) at fermi level (Brihuega et al. 2011), in agreement with Anderson criterion (Reich et al. 2003). From the application point of view, J_C and upper critical field (H_{C2}) are of utmost importance in granular type II superconductors. According to WHH (Werthamer–Helfand–Hohenberg) theory (Werthamer et al. 1966),

$$H_{C2} = 0.69 T_C \frac{4ecK_B}{\pi} N(0)\rho_N \quad (\text{in “dirty limit” } L_{\text{eff}} \ll \xi_0)$$

L_{eff} – means free path

ξ_0 – intrinsic coherence length

e – electron charge

K_B – Boltzmann constant

$N(0)$ – DOS at fermi level

From above it is clear, that with a decrease in size and, in turn, increasing disorder and hence large ρ_N , H_{C2} would monomerically increase, provided T_C and $N(0)$ do not decrease with decreasing size. With a decrease in particle size both $N(0)$ and mean free path L_{eff} decreases. A decrease in $N(0)$ would reduce H_{C2} , whereas decrease in L_{eff} would enhance H_{C2} . The above formula forms the basis for increasing H_{C2} by introducing non-magnetic impurities or by preparing nano superconductor provided T_C does not change appreciably.

Biopolymers are effective sequestering agents for metal cations to prepare nanoparticles. Biopolymers form growth – restricting “cocoon” during calcination process. When polymer is taken as a template agent, its concentration is important for controlling the shape (Alikhanzadeh-Arani et al. 2014) (Hall 2006). Different sizes and morphologies result from using different amounts of polymer because of nucleation and embedding of nano particles in the polymer matrix. There are only a few reports on the size dependence of critical magnetic fields in superconductors, and these are mostly

on typical elemental superconductors viz., Al and Pb (Bose et al. 2006). Biopolymer-mediated synthesis of high T_C superconductor is evolving into an important research area with recent attention toward the sol-gel method in presence of biopolymer likes, chitosan (Alikhanzadeh-Arani et al. 2012), sodium alginate (Schnepf et al. 2008), and dextran (Walsh et al. 2007) (Wimbush et al. 2012). Researchers have successfully prepared superconducting nanowires (Hall 2006; Zhang et al. 2012) porous superconducting foams (Walsh et al. 2009) and ordered porous bodies using biopolymers during the sol-gel method. Biopolymer-mediated synthesis of superconductors could overcome difficulties in the preparation of long superconducting tapes.

Accordingly, there are reports of (i) an enhancement in H_{C2} of Al (Cohen and Abeles 1967) and $PbMo_6S_8$ (Niu and Hampshire 2003) with decrease in grain size, by 60% and 100%, respectively; (ii) high yield of superconducting nanoparticles without considerable change in T_C , even with high Ir addition in Y123 (Wimbush et al. 2012); and (iii) improved synthesis time of $LuBa_2Cu_3O_{7-\delta}$ of 48 h compared to 500 h for solid-state synthesis method (Dedman et al. 2010a). At the same time, none of these systems showed a decrease in T_C .

Sol-gel synthesis of superconductor using biopolymer also facilitates easy introduction of dopant (Walsh et al. 2007). Walsh et al. (2007) have studied the effect of varying Ag concentration on J_C and demonstrated silver as a flux pinning agent to enhance J_C in Y123. Walsh et al. (2007) have demonstrated controlled crystal morphology in Y123 by incorporating biopolymer into sol-gel synthesis protocol (Table 1). These authors (Walsh et al. 2007) have shown the different morphologies: (i) sponge like when prepared with biopolymer; and (ii) typical poly dispersing in Y123 prepared without dextran. Authors have attributed the observed high J_C to its small crystallite size as well as the surface pinning of the porous structure.

Self-sacrificial biopolymer templates can be used to create superconducting sponges. These sponges exhibit higher thermal shock resistance with comparative uniform cooling (Noudem et al. 2004; Noudem et al. 2003), such sponges are of practical interest for their application as fault current limiter (FCL) superconducting bearings.

Sr. no.	Biopolymer	Detail about biopolymer	Superconductor prepared using biopolymer	Application of biopolymer
1.	Ethyl cellulose nanoparticles	Ethyl cellulose is a derivative of cellulose. Here, ethyl groups replace hydrogen in the hydroxyl group of glucose residues	Gd $Ba_2Cu_3O_{7-\delta}$ (Gd123) (Alikhanzadeh-Arani et al. 2014)	Used for: <ul style="list-style-type: none"> 1. Hydrophobic film coating (Porter 1989) 2. Masking an unpleasant taste 3. Controlled release coating of soil dosage forms (Interrante et al. 1988)

(continued)

Sr. no.	Biopolymer	Detail about biopolymer	Superconductor prepared using biopolymer	Application of biopolymer
2.	Polysaccharide dextran derived from bacteria <i>Leuconostoc</i> spp.	Dextran is a complex branched polysaccharide. It consists straight chain of (α -1,6) glycosidic and a majority of (α -1,3) branches	A. $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ (Y123) (Walsh et al. 2007, Wimbush et al. 2012)	Calcination in the air is done to foam the dextran. Thus, sparging the rapidly oxidizing synthesis mixture and hence controlling overall morphology and crystal growth
			B. Ir doped (Y123) (Wimbush et al. 2012)	
			C. $\text{LuBa}_2\text{CuO}_{7-\delta}$ (Dedman et al. 2010)	
3.	Carrageenan	Carrageenan is plant derived biopolymer. Kappa-“Carrageenan consists of repeating disaccharide units of alternating ($\frac{1}{3}$) – α -D-galactose-4-sulfate and ($\frac{1}{4}$) – β -3,6-anhydrous-D-galactose residues joined in a linear chain” (Walsh et al. 2009)	4BCO (Walsh et al. 2009)	–

Synthesis of Nanoparticles Through Biopolymer Template

Nanoparticles are particles in the range of 1–100 nm and typically below 10 or 20 nm. Richard Feynman introduced the concept of nanomolecular level in the year 1959 through his famous talk titled “There’s plenty of room at the bottom” (Richard 1960). Since then, researchers have found wide applications of nanoparticles in various fields such as medicine, therapeutics, dye treatment (Sheshmani et al. 2014), photocatalysis, water treatment, and many more. The reaction conditions and precursor materials determine the shape, size, and physiochemical properties of the nanoparticles (Dedman et al. 2010). Besides, the use of solvents and reducing agents during synthesis impact environmental sustainability due to their non-biodegradable and toxic nature (Sheshmani et al. 2014). As the technological advancement of society cannot be stopped, it is important to develop synthesis procedures that are simple and environmentally benign (Hu et al. 2010). In this direction, biopolymers have shown promising results to be used as capping/ stabilizing and reducing agents for the synthesis of nanoparticles.

Biopolymers are either used as stabilizers and capping/reducing agents in the synthesis of nanoparticles. The morphology of gold nanocrystals is controlled using

bovine serum albumin (a single polypeptide chain of 583 amino acids) in combination with a small amount of silver nitrate additive to form Au octahedrons with an average edge length of 73 ± 9 nm. Normally, polymers containing carbonyl and tertiary amine groups such as poly (vinyl pyrrolidone) play an important role in the regulation of the shape of noble metal nanomaterials. The amide groups present in BSA protein act as surface regulating polymer like multiple tertiary amides of PVP. Cui et al., reported a method of synthesis of nanoplates in which hyaluronic acid (polysaccharide based natural biopolymer) acts as both a reducing and stabilizing agent. This study focuses upon differences in the shapes and morphology of nanoparticles led by aging of HA and AgNO₃ solution employed in the synthesis. Interestingly, a distinct morphology of spherical NPs is obtained using fresh solution and a morphology of triangular nanoplate using an aged solution in a short duration of 15 min (Cui et al. 2008). In another strategy, surfactant free PLGA nanoparticles are made to avoid the extra purification step for removing excess surfactant. It is also important to effectively replace the role of surfactants (zwitterionic, cationic, anionic, and nonionic) in controlling the structure of nanoparticles in aqueous phase. This is achieved by grafting Alkaline lignin (ALGN) and lignosulfonate (SLGN), two forms of lignin (hydrophilic natural biopolymer), upon poly (lactic-co-glycolic acid) (PLGA, a hydrophobic synthetic polymer) using covalent interactions to form (A/S)LGN-PLGA biopolymer template. The ALGN-PLGA nanoparticles are made by self-assembly and emulsion-evaporation process. The diameter of the synthesized nanoparticles ranged from 100 to 200 nm. The varied LGN/PLGA ratio was an important factor in controlling the shell thickness and core size of the synthesized nanoparticles (Astete et al. 2020).

In this section, we discuss the application of chitosan, cellulose, sodium alginate, and hemicellulose to assist the synthesis process of nanoparticles.

Chitosan

Chitosan is a class of semisynthetic polymers originated from the alkylation of chitin in its natural form in crustaceans. It is a copolymer of β -[1 \rightarrow 4]-linked to *N*-acetyl D-glucosamine and D-glucosamine units. Being a non-toxic biodegradable polymer, chitosan polymer is being explored for various applications including templated synthesis of nanomaterials. Sheshmani et al. (2014) reported a facile chemical route for the synthesis of nanocomposite graphene/Fe₃O₄/chitosan using the solvothermal method. The composite was used as a nano adsorbent material for dye degradation of acid orange-7 dye in wastewater treatment. Ceria nanoparticles were synthesized using chitosan as a template to obtain porous ceria-chitosan nanospheres with an average size of 4 nm and BET specific surface area of 105 m²g⁻¹. It was concluded that stable residue of the nanoparticles can be obtained after two weight loss steps owing to the evaporation of water and decomposition of chitosan (Sifontes et al. 2011). Chitosan/chitin film has been used as a seed for silver nanoparticle synthesis. These silver nanowires were used for the synthesis of nanowires using the drop cast method (Chandran et al. 2018). Similarly, tungsten nanoparticles can be synthesized

using chitosan as template. In this technique, ammonium metatungstate was encapsulated in chitosan biopolymer using calcination at temperature within 400–600 °C. The nanoparticles produced using this reaction were used for electrochemical hydrogen evolution and showed four times higher activity than bulk WO_3 (Ganesan and Gedanken 2008).

Navarro et al. reported a synthetic procedure using chitosan for preparing a hybrid nanostructure with narrow particle size distribution (1.2–8 nm). Magnetic nanoparticles of MFe_2O_4 (M-Mn or Mg) were synthesized from the polymeric solid solution of cations chelated within a chitosan matrix (Garza-Navarro et al. 2010). Hu et al. reported a method of synthesis of chitosan-acetic acid nanoparticles, wherein, the ratio of chitosan and acetic acid played an important role in controlling the size of the nanoparticles. The synthesis was done by polymerization of the monomer using acetic acid. In addition to the content of the chitosan, the role of pH was also investigated and a correlation between the pH/content of chitosan and the size of nanoparticles was reported (Hu et al. 2010).

Cellulose

Cellulose is the most abundant biopolymer in nature obtained from sources such as plants, animals, algae, and fungi. The plant originated cellulose is accompanied by small quantities of hemicelluloses and lignin. The applications of these polymers in the synthesis of nanoparticles are discussed further. The cellulose structure contains D-glucopyranose ring units in the 4 C1-chair configurations linked via β -1,4-glycosidic bonds (Heinze 2015). Cellulose biopolymers are used as scaffolds to facilitate the seeding of nanoparticles. However, they also facilitate the synthesis process. On a similar front, Li et al. reported the synthesis of spindle-shaped TiO_2 nanocrystals. These crystals showed high efficiency heavy metal adsorption (Li et al. 2015). Padalkar et al. used cellulose nanocrystals (isolated from tunicates) to synthesize a series of metallic nanoparticles: Ag, Au, Cu, and Pt. The synthesis was done in three steps: extraction of cellulose; sulfuric acid hydrolysis of cellulose pulp (for obtaining sulfate-functionalized tunicate CNCs); followed by sulfation (Zhong and Jin 2009) (Padalkar et al. 2010). Along with synthesis, the usage of cellulose also plays an important role in controlling the morphology of the nanoparticles. Liu et al. (2012) reported a method of using regenerated cellulose films as a template to synthesize monodispersed Fe_2O_3 nanoparticles, achieving an average particle size from 28 to 42 nm without a size selection process.

Sodium Alginate

Sodium alginate can be used as a template during synthesis to reduce the toxicity of nanoparticles. Gao et al. (2008) prepared Fe_3O_4 nanoparticles using sodium alginate as a reducing agent in a redox based hydrothermal synthesis. The reaction was carried out using $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and urea as starting materials. The average size

obtained was 27.2 nm. Similarly, Kuzmanović et al. synthesized manganese doped cadmium sulfide nanoparticles of diameter 4 nm using a sodium alginate template. The doping of manganese with cadmium sulfide was present throughout octahedral sites. The chemical synthesis led to the chemical bonding between Cd^{2+} ions and alginate showing low toxicity as compared to CdS nanoparticles. These particles were reported to have applications in bioimaging due to the distorted crystal field instigated by manganese showing high photoluminescence properties (Kuzmanović et al. 2017).

Alginate based TiO_2 nanospheres were reported in 8–11.6 nm range according to the synthesis temperature ranges (Dutta et al. 2012). These particles showed high photoconductivity against the dye, Rose Bengal, due to enhanced photon to electron conversion.

Hemicelluloses

Hemicelluloses are one of the most abundant polymers present in the cell wall of plant cells, covalently linked with lignin and physically intermixed with cellulose. Hemicelluloses are complex heteropolysaccharides made up of xylose, mannose, galactose, arabinose, uronic acid, and glucose. Hemicelluloses consist of reducing aldehyde groups and hydroxyl groups and may exist as random coil chains in aqueous solution or helical chains in aqueous solution (Mazeau and Charlier 2012). The hemicelluloses from bamboo along with glucose are used to synthesize silver nanoparticles by reducing silver nitrate in an aqueous medium. The presence of hydroxyl groups on the hemicellulose polysaccharide matrix enables the complexation of silver ions with strong non-covalent interactions. The aldehyde groups of the glucose reduce the silver ions to elemental silver causing nucleation of silver and formation of nanoparticles. Also, the helical structure of hemicelluloses prevents agglomeration of silver nanoparticles. However, at higher concentrations of silver nitrate, hemicelluloses are not able to effectively cap silver nanoparticles resulting in nanoparticle aggregation (Peng et al. 2013).

Green Solvents Can Make Biopolymer Assisted Material Synthesis More Environmentally Sustainable

Green chemistry has revolutionized the field of material science which in retrospect consists of large-scale industrial synthesis of materials using environmentally toxic solvents, chemicals, and reagents. The use of aromatic chlorinated solvents (toluene, benzene, phenol, etc.) causes ozone layer depletion, results in environmental toxicity, and requires appropriate alternatives like biodegradable ionic liquids (ILs) to ensure a greener synthesis process. Several nanomaterials including gold, silver (Wang et al. 2016), copper (Gawande et al. 2016), and superconducting materials like MgB_2 (Xu et al. 2008) are synthesized using the aromatic chlorinated solvents like toluene.

The solvents used in the material synthesis processes significantly contribute to environmental pollution necessitating a strong need for green solvents with properties such as low environmental toxicity, low vapor pressure, biodegradability, and high boiling point. Green solvents with such properties include polyethylene glycols (PEGs), water, ILs, and supercritical (SC) fluids. ILs are organic salts, composed of a polyatomic inorganic anion and an organic cation, and are commonly preferred green solvents. ILs possess environmental compatibility along with good operational safety. They are environmentally benign with negligible vapor pressure and suitable to be used as a solvent in sustainable synthesis processes. There are notable pursuits of using green solvents for NP synthesis, for instance, imidazolium IL that has been used to synthesize metal nanoparticles such as silver and gold. The long alkyl chain of imidazolium enhances the stability of AuNPs, lowers aggregation, and increases solubility (Safavi et al. 2012). Imidazolium based IL acts as both stabilizer and solvent for the nanoparticle synthesis process. Silver nitrate (AgNO_3) is reduced to AgNPs by imidazolium halide ionic liquids along with H_2 (hydrogen gas). A significant advantage of this synthesis process is that it does not require the removal of excess reducing agents left in the reaction system (Patil et al. 2020). ILs are faced with the limitation of structure- biodegradability relationship in which pyrrolidinium ionic liquids are more readily biodegradable than imidazolium ionic liquids (Neumann et al. 2014). Glycerol, also called “Organic Water,” provides several advantages besides being a green solvent such as immiscibility with aliphatic hydrocarbons, solubility with organic and inorganic compounds using hydrogen bonds, and polar nature. The concentration of glycerol controls the size of AuNPs in the green synthesis procedure without involving toxic chemical reagents.

Synthesis procedures of materials based on traditional chemistry approaches do not minimize hazardous environmental impact and also require high energy instrumentations. The synthesis of advanced materials (nanoparticles and superconductors) using physical and chemical methods involves the use of hazardous chemicals, reagents, and solvents (Zhang et al. 2020). Besides, these synthesis processes are associated with high energy consumption (Horwat et al. 2011) like pulsed laser deposition of $\text{LuBa}_2\text{Cu}_3\text{O}_7$ and $\text{LuBaSrCu}_3\text{O}_7$ single-phase superconducting thin films (Schwab et al. 1993) and pulsed laser deposition of oxide nanoparticles (Oxide NPs: Y_2O_3 , Gd_2O_3) (Amans et al. 2011). The physical methods also comprise high temperature and pressure conditions such as spark plasma sintering based synthesis of high-density MgB_2 (Dadiel et al. 2021) and Co/Ni-doped BaF_2As_2 superconductors (Zaikina et al. 2018) consisting of step-wise exposure of high pressure and temperature conditions. In chemical methods, expensive and environmentally toxic stabilizers (surfactants like CTAB), organic solvents (aromatic hydrocarbons, chlorinated solvents), and reducing agents (sodium borohydride) are used. Green chemistry altogether attempts to minimize the environmental impact of the overall reaction system so that the economic feasibility and environmental sustainability does not limit the synthesized material’s translational application. Besides, the use of biopolymer-assisted growth of superconductors and nanomaterials should be combined with an appropriate choice of a green solvent.

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Nature-Inspired Biomimetic Polymeric Materials and Their Applications

48

Sherin Antony, T. R. Anju, and Bejoy Thomas

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S. Antony (✉)

Department of Zoology, Bharata Mata College, Thrikkakara, Kerala, India

e-mail: sherin@bharatamatacollege.in

T. R. Anju

Department of Biotechnology, Newman College, Thodupuzha, Kerala, India

e-mail: anju.tr@newmancollege.ac.in

B. Thomas

Department of Chemistry, Newman College, Thodupuzha, Kerala, India

e-mail: bejoy.thomas@newmancollege.ac.in

Abstract

Biomimetics, an interdisciplinary field of science denoting synthetic methods which mimic biochemical processes in nature, has contributed to the invention of many novel technologies and designs. In this chapter, we discuss the various nature-inspired biomimetic polymers, its advantages and limitations, applications, and future prospects. Classification of biomimetic materials is broadly based on function, process, structure, and molecular aspect of material, which is the source of biomimicry. Biomimetic materials find its applications in various fields like architecture, robotics, superhydrophobic, self-cleaning materials, adhesives, nanotechnology, and biomedical. Biocompatible and bioactive substances from biological sources fulfill various functions that are critical for the sustainability of life. Significant research based on architecture of biological materials and their tissue interaction has been carried out toward engineering revolutionary bioinspired structures that show superior biocompatibility and bioactivity. Advancements in biomimetic research makes way for the creation of novel nature inspired biopolymers which sustainably meet design challenges.

Keywords

Biomimetics · Biopolymers · Therapeutics · Tissue engineering

Introduction

Scientists and inventors study characteristics of things in nature and come up with amazing technologies and products invented as a result of studying nature. Biomimetics, an interdisciplinary field of science relating to or denoting synthetic methods which mimic biochemical processes in nature, has fascinated humans for generations and inspired the invention of many novel technologies and designs.

Biomimetics is defined as the study of biologically synthesized materials and biological mechanisms and for the purpose of reproducing analogous products by artificial means which imitate natural ones, and the investigation of nature and its models to emulate, in order to solve human problems (Fratzl and Weinkamer 2007). Parallel terms include bionics and biomimicry. As Vincent et al. (2006), quote, “The term biomimicry, or imitation of nature, is defined as copying or adaptation or derivation from biology” (Vincent et al. 2006).

The term “biomimetics” originates from the Greek words “bios” and “mimesis” which decodes as “life” and “to imitate,” respectively. In 1969, Otto H. Schmitt devised the term biomimetics (Schmitt 1969). This emerging field of biomimetics is multidisciplinary and has advanced as new technologies based on biologically inspired engineering at infinitesimal levels. The sustainability basis of this field is that nature follows the path of minimum expenditure of energy, utilizing the most common materials to achieve a task.

Innovative Products from Nature-Inspired Biomimetics

Biomimetics is not a new idea – historiographic considerations of the science of biomimetics clearly manifest that this has been practiced much earlier. “*Codex on the Flight of Birds*,” inscribed by Leonardo da Vinci in 1505–1506, ventured that air travel by humans could be modeled on avian flight mechanics (Zanon 2009). In 1903, the first successful airplane flight by the Wright brothers occurred. The Wright brothers modeled the wings’ control mechanism based on the technique birds uplift and change direction manipulating air currents. In 1851, landscape designer Joseph Paxton built the Crystal Palace for the Great Exhibition, the first international expo of manufactured products in London. The palace’s unique architecture with crisscrossed iron girders, to support nearly 300,000 panes of glass, was inspired by the leaf of a water lily, where interconnected ribs aid the plant support substantial mass in water. Swiss engineer George de Mestral patented Velcro in the year 1955. During a hunting trip in the Alps, his dog became covered in burs. Cockleburrs got gripped to clothes readily due to hooks. Mestral found that their hook-and-loop construction, can be gripped and ungripped with force. Inspired by the seeds’ tiny hooks, de Mestral proposed a fabric product: one with hooks and the other with loops (WIPO 2016). In 1960s, when NASA used Velcro in space shuttles to prevent food, equipment, and other items from floating away in zero gravity, it became widely known.

American biophysicist Otto Schmitt (1969) used the term “biomimetics” (Patterson 2009). No stranger to bioinspired technology, Schmitt had invented an electrical circuit modeled after the neural impulse systems of squids in 1934. Bioinspired technology-driven Schmitt, stirred by neural impulse systems of squids, had invented an electrical circuit in 1934.

A technology that resembles the grooves found on shark skin was tested by NASA in 1986. Inspired by self-cooling mounds of African termites in 1996, Architect Mick Pearce created the Eastgate Centre in Harare, Zimbabwe. The building uses chimneys that draw in cool air to maintain a temperate environment as it does not have mechanized heating and cooling system. The ventilation costs one-tenth of that in a comparable, air-conditioned building. Also, it uses less energy (35%) (Scobey-Thal 2014).

In 2006, Richard Bonser published a study that assesses the growth of biomimetic innovation (Bonser 2006). Accordingly, between 1985 and 2005, the number of patents worldwide containing the word “biomimetic” or “bio-inspired” augmented by a factor of 93, which is supremely high. The United States-based firm, Biomimicry 3.8, consults for and empowers companies about ways to incorporate bioinspired innovation into their practices (Scobey-Thal 2014). In 2012, Sorensen and Galvez, founders of NBD Nanotechnologies United States, produced a successfully tested concept for a water bottle which was inspired by the Namib Desert beetle. Like the insect, which draws water from air by collecting condensation on microscopic bumps on its back, the bottle would harvest air moisture. The device could store up to 3 liters of water every hour (Scobey-Thal 2014). In 2014, an article in *Nature*, on metal-free battery, was published on carbon-based molecules called

quinones (naturally abundant) analogous to those that accumulate energy in animals and plants (Huskinson et al. 2014). This technology delivers the accumulation of renewable energy in large grid systems.

Humans have been turning at nature for being guided for answers to problems throughout their lives. One such example is the honeycomb structure of the beehive. Bees and wasps build nests which are made of hexagonal cells, both space saver and strong. Stacked together, hexagons fill spans in an offset arrangement with six short walls around each “tube,” giving structures high compression strength. Beehives are efficient in dissipating heat well, which prevents the waxy structure from melting on hot days. Efficiency, strength, and controlled heat loss are all important for human structures as well, so it is no wonder that honeycombs inspire human design. Structures inspired by honeycombs in bee nests are widely used to build airplane wings, light-weight building materials, flexible panels for bridge construction, and packaging and acoustic dampening materials.

Other examples of biomimetics include the development of superhydrophobic surfaces based on the lotus leaf, the “shark skin” drag-resistant swim suit (Hwang et al. 2015), and Velcro fasteners modeled on burrs (Velcro 1955). Shape of airplanes developed from the design of birds, wings, and their feathers – was the result of numerous years of biomimetic research on the structure and design of birds.

The whole idea of this chapter is a detailed discussion about the various biomimetic materials used, its advantages and limitations, applications, and future prospects.

Biomimetic Materials: General Classification

The symbiotic relationship between humans and nature has been crucial in our coexistence in this world, and this science of biomimetics opens up many examples for innumerable applications. Designing innovative products by the application of the rules of biomimetics plays a significant role in industries where it offers human convenience in inter disciplinary fields (Benyus 2009). Many researchers pointed out the significance of understanding these examples for providing revolutionary applications using the science of biomimetics.

Bioinspired materials have attracted a lot of attention in the recent decades with an increased interest in identifying nature-refined biological products that can be used as a source to develop innovative advanced materials. The excellent mechanical and functional properties exhibited by biological materials are coincidentally important in many engineering industries. The fundamental aspect in many of these biological materials, which are simple in composition but efficient in performance, is contributed by the hierarchical structure, i.e., the organization of building blocks. Being durable, reliable, and nontoxic, these materials present inspirations for designing advanced materials applying biomimetics (Wang et al. 2020).

Glaser and Viney (2012), reported that the character of new biomimetic materials lies in understanding the structural and functional aspects of hierarchical structures in nature and remodeling them for our use. Most of these hierarchical structures were

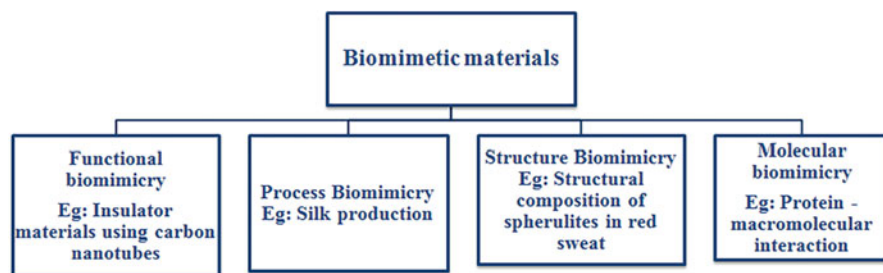


Fig. 1 Schematic illustration of the broad classification of biomimetic materials based on the biomimimery which has inspired the production of the (bio)materials

constructed from small building blocks which later on attain a complex polymeric structure, and fine-tuned by adaptation and optimization to yield excellent performance.

A typical example is the bone toughness which is extraordinary, due to the presence of collagen fibrils at micro/nanometer levels, which also ensures the assembly of several types of bones with varied functions (Fratzl et al. 2004). The changes in functionality of a material can be attained by hierarchically structuring a material in different ways, rather than finding a new material. Many examples in nature inspire researchers to discover new materials which mimic nature and adapt their properties for a wide range of applications.

To throw more light on the science of biomimetics and nature-inspired biopolymers, it would be convenient to differentiate between various classes of biomimimery, on the basis of which natural trait is being imitated (Fig. 1). Classification of biomimetic resources can be done broadly based on functions, process, structure, and molecular aspect of materials, which is considered as the source of biomimimery.

Functional Biomimimery

Physical and/or chemical properties of a biomaterial are inspired from natural sources which can be exploited for making a biomimetic material. Functional biomimimery concentrates on acquiring information from the way living things execute specific functions which can be extrapolated in making a new biomimetic material. In every successful biomaterial, there is some element of functional biomimimery (Glaser and Viney 2012).

An example for functional biomimimery is the development of insulator materials mimicking the functionality of the fur of polar bear which is hollow and keeps them warm. Functionally, bear's fur insulates or conserves heat provided by its features. This property of fur is used as a model to create an insulator (Cui et al. 2018). Inspired by the polar bear hair, waterproof and lightweight, thermal insulating carbon tube material is created. It finds application in outerwear and in insulated buildings. The evolutionary adaptation of polar bear to prevent heat loss in cold and humid conditions has turned out to be an excellent model for engineers to design synthetic heat insulators (Cui et al. 2018).

Another aspect of functional biomimicry is when a natural material is taken from an organism and replaced for damaged material in a host. A simple example for this is provided by false teeth made from ivory of elephant, walrus, or hippo in earlier times, which were used to make dentures.

Process Biomimicry

In process biomimicry, a natural process is copied, like photosynthesis in the leaf of green plants. Photovoltaic systems were created which takes up solar energy, the first footstep at imitating a leaf which uses up energy (Benyus 2009). Research is undergoing to make highly efficient solar cells that imitate nature as artificial leaves – water-gel-based – that combine plant chlorophyll with carbon materials, resulting in cost-effective and flexible solar cell (Mcclure 2019).

Many invertebrate animals like spiders, several insects, and myriapod species (an arthropod group that includes centipedes, millipedes, and related animals) use the silk they produce for varied essential functions, for their survival, i.e., prey capture, for habitat construction, and for egg protection. Adult weaver ants which live in trees are known for their unique nest-building action where workers construct nests by weaving together leaves which are glued together by larval silk. The natural silk made by spiders and some insect larvae is turned from aqueous solution, and it forms a water-insoluble final product. This alteration in solubility is accompanied by changes in the conformation of the integral protein chains, but the chemical nature of the chains remains unaffected. As quoted by Viney (1997), “The water-soluble protein molecules secreted by the silk glands are globular; they aggregate into a processable liquid crystalline fluid within the ducts leading from the glands to the spinnerets, and thence undergo shear nucleated reorganization into a solid fiber stabilized by water-insoluble crystal.” Nature thus provides a proposal for sustainable and environmentally gentle dispensation of polymer fiber.

Biomimicry in construction is the practice of designing buildings and products that simulate processes that occur in nature. In simulating process that is shown by natural materials, a good example is the manufacture of wood glue which is formaldehyde free and nontoxic, inspired by the way blue mussels firmly attach under the water using flexible, thread-like tentacles (Messersmith 2013).

Structure Biomimicry

Structure means “the arrangement or formation of the tissues, organs, or other parts of an organism.” Structure biomimicry ensues once nature inspires the atomic and molecular association at nano/micro structure levels in a biomaterial. However, Darwin’s Principle of “natural selection” plays inevitable role making nature’s choice an accepted model, and this has been tested and proved to be the best choice in the long course of evolution and survival. While designing biomimetic material, a perfect understanding of the polymeric blends is essential to attain the structural

integrity of the nature's model. Visual inspiration in architecture is a more easily adaptable mode of structure biomimicry, for example, a "picture of sea turtle shell can be used to shape a reinforced concrete for a large span roof structure in an exhibition building" (Kshirsagar 2017).

Nature is skillful in achieving multifunctionality, by the use of hierarchical structure, and thereby optimizing many characteristics in its building block. This permits autonomous control of properties that are a result of structure at different dimensions. The best example of multiple action and thereby function is by the "red sweat" (red-colored oily secretion) of Hippopotamus (Reed et al. 2009). "The fluid sweat contains two types of liquid crystalline spherulite (spherulites (from Greek sphaira = ball and lithos = stone) are spherical semicrystalline regions inside non-branched linear polymers)." Some of the spherulites are efficient in light scattering due to their structural characteristics so that the sweat shows sunblock property; the other spherulites, which are coarser at microlevel, provide the characteristics to spread on skin due to lessening of viscosity (Saikawa et al. 2004). Both these assets of red sweat along with molecular biomimicry of the red sweat are useful in the making of better skincare products that are potentially useful as sunscreens (Reed et al. 2009).

Another instance of structure biomimicry can be observed in spider's silk; the fibers form the "major component of the drag line and the radial threads of a web" are secreted by major ampullate glands (Trancik et al. 2006). Silks are natural polymers consisting mostly of the amino acid, alanine, serine, and glycine, and are produced by arthropods in the class of Insects, Arachnida, and Myriapoda (Bini et al. 2004). Biologically, silk fibers provide structural support, defense, and assist in food capture. The nanostructure reveals "a bimodal size distribution of crystalline regions" which consists of larger and smaller crystals. "The larger crystals promote the high initial stiffness of the fiber when it is deformed in tension; however, their imperfect internal structure prevents them from acting prematurely as stress concentrators that could accelerate failure. The smaller crystals do not contribute significantly to initial stiffness, but they provide stability to the material during plastic deformation" (Turner and Karatzas 2004). Biomaterials mimicking this microstructure of spider silk which shows optimal combination of mechanical properties could have tremendous applications.

Molecular Biomimicry

Molecular biomimicry happens when a biomaterial is produced by atoms and molecules available from natural sources. A typical case is that of "filamentous bacteriophage," due to its adaptability, modifying potential, and sturdiness as a promoter of liquid crystalline phase formation (Reed et al. 2009). This property aids in self-assembly of building blocks in a bottom-up approach. Filamentous phage or genetically engineered phagemids (a DNA-based cloning vector, which has both bacteriophage and plasmid properties), at the right phase of its life cycle, can guide the self-assembly of complex structures in perfect symmetry and alignment. "Genetically modified fusion phagemid accomplishes molecular biomimicry by stabilizing liquid crystalline order in the same way that a native filamentous phage would" (Reed et al. 2009).

These filamentous phages are biodegradable, and thus used as a disposable constituent of the repaired tissue. “Recombinant filamentous bacteriophages are successfully encapsulated in biodegradable polymeric microparticles (MP) for stimulation of innate and adaptive immune responses.” Microparticles (MPs) are made of poly (lactic-co-glycolic acid) (PLGA) to encapsulate bacteriophage and retain their immunogenic properties. Bacteriophage-encapsulated PLGA microparticles serve as a vehicle for vaccine delivery (Jamaledin et al. 2020). Molecular biomimetics is thus an evolving field in which nanotechnology along with molecular biology principles can be hybridized to provide solutions to scientific problems.

Applications of Biomimetic Materials

Biomimetic materials show similar characteristics with biomaterials and are modeled with a means to reproduce the functions and traits of a material that is naturally produced by living organisms. The principles and accomplishment of a biomimetic material depends on the restoration of natural structure and function which is environmentally stable. Biomimetic materials find their applications in various fields like architecture, robotics, superhydrophobic, self-cleaning materials, adhesives, nanotechnology, and biomedical applications (Fig. 2).

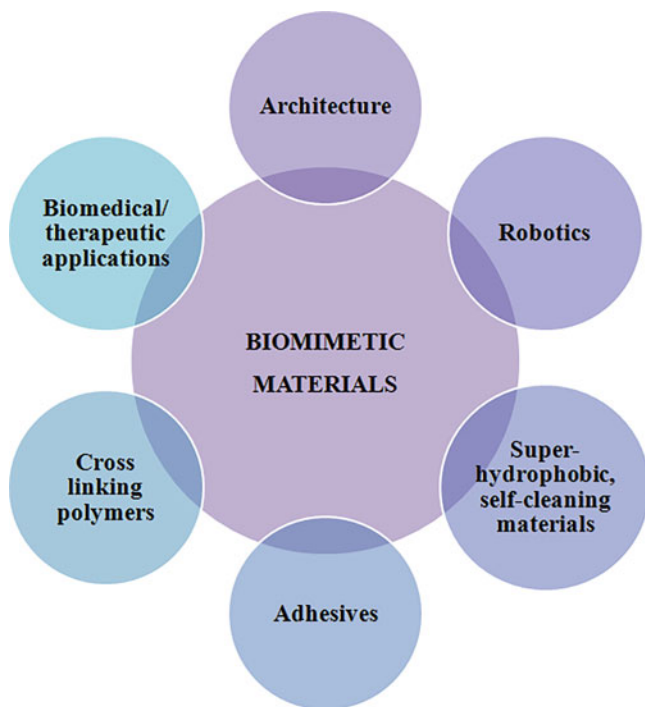


Fig. 2 Schematic representation of the applications of biomimetic materials

Biomimetics in Architecture

Biomimetic architecture seeks sustainable solutions to resolve architectural problems concerning building various structures. Understanding the principles governing the inspiring natural forms or natural materials will provide immense source for sustainable designs. One such example is buildings designed mimicking the hierarchical structure of organic bone which can withstand tremendous force of earthquakes. Biomaterials like bone and teeth are nanocomposites with a hierarchical structure that shows excellent mechanical properties. This is because bones are produced by composites in complex, layered structures with ordered architecture that spans nanoscale to the macroscale (Meyers et al. 2008). This structural composition provides great high mechanical strength, which can be mimicked in the construction of buildings.

Another example is Gecko's Climbing feet-inspired climbing pads. Gecko's feet have minute hair coverings on their toes which aid to climb up any vertical surface with great grip that prevents fall off (Arzt 2006). This biomechanics of feet of gecko is imitated in making a pair of climbing pads that support human weight. Those climbing pads are layered with polymeric adhesive tiles and covered with an array of polydimethylsiloxane (PDMS) and are attached to an independent degressive elastic element and are sawtooth-shaped (Hawkes et al. 2015). When these pads, which are about the width of a human hair, are pulled upon, they create adhesion forces (Fig. 3a).

Another occurrence of application of biomimetic architecture for human use is the bullet train made by Japan scientists which model was inspired by the shape and sleekness of the kingfisher. This led to upgradation of bullet trains which showed reduction in tunnel boom ("irritating noise created by these trains when they displace the surrounding air") and thereby aided in avoiding tunnel damage. The overall aerodynamics needed to be modified, which made the train more streamlined, inspired by the beak of the Kingfisher bird (Fig. 3b).

Humpback of whales provided the design for highly efficient wind turbines. Propulsion for steering in water is done by bumpy tubercle fins. Biomimetic fins ("a flattened appendage on various parts of the body of many aquatic vertebrates, including fish and cetaceans, and some invertebrates, used for propelling, steering, and balancing") reduced the drag and also improved lift. Another versatile application is antimicrobial film which emulated shark skin. The skin of a shark is covered with dermal denticles which are like minute teeth creating an antimicrobial zone. This biomimetically acting film coating prevents growth of microorganisms and other marine life on ship hulls. These dermal denticles create low-pressure zones which reduce the drag during motion, and this design of sharkskin is incorporated in swimsuit design (Wen et al. 2014).

Another example is that of Woodpeckers, which are excellent excavators and are also known for their shock-absorbing beak. As the woodpeckers strike the wood, the bird's head shows shock-absorbing phenomena. This inspired the making of shock-resistant black boxes and micrometeorite-resistant spacecraft (Lee et al. 2014).

Termite mounds are another model of biomimetics in architecture. They are huge structures built by small insects and offer temperature-stabilized habitats that protect

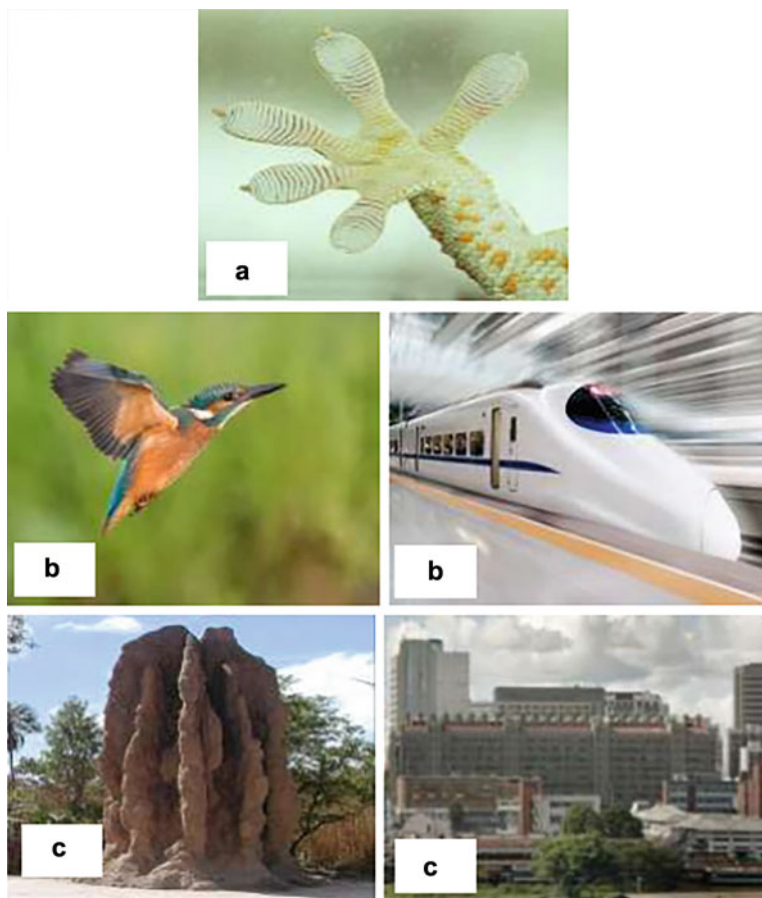


Fig. 3 Examples of nature-inspired ideas in architecture. (a) Gecko feet. (b) Kingfisher beak (left), Bullet train (right). (c) Termite mound (left) and mound inspired building (right) in Eastgate Centre, Harare Zimbabwe

the organism from extreme environmental fluctuations and allow them to cycle energy and matter, and communicate information with the world outside. Termite mound-inspired ventilation system works on the principle of convection. Termites are experts in modeling an intricate ventilation system for cooling, which consists of air pockets. Buildings have been designed mimicking the termite mound, which maintains temperature without any mechanical systems (King et al. 2015) (Fig. 3c).

Biomimetics in Robotics

Biological intelligence and the field of biomimetics in robotic science show advanced trends toward successful research. Robots, are synonymous with synthetic

animals with senses, intelligence, perception, and motor skills desired to operate in the world in ways that are highly beneficial for human use. As Wang et al. (2021), defined, “Biomimetic robotics is the development and integration of biomimetic mechanisms by imitating biological structures or behaviors.” Biomimetic robotics find wide range of application where robots are created with characteristics seen in organisms and functions, such as “humanoid robot in the home environment, quadruped robot in the field, and bird-like flying robot in the sky” (Wang et al. 2021). A crucial feature of biomimetic robots is their competence to acclimate to the natural environment. Bioinspired intelligent control systems work in robotics to physically interact with the world.

Biomimetic robotics are empowered by biomimetic intelligence, which aids it to be exploited for practical purposes. “The biomimetic multi-legged robot simulates the structure of living creatures’ legs and the way they move, such as human-like biped robot, tetrapod-like quadruped robot, and spider-like hexapod robot. The advantage of the multi-legged robots is their adaptability to various terrains which can overcome the site constraints so that they are suitable for tasks like search-and-rescue and environment detection” (Shigemi et al. 2018).

The “biomimetic flapping-wing robot” emulates the flight principles of birds and insects in nature which use their “fast-vibrating wings” to perform soaring, revolving, and other movements shown by birds and insects (Wang et al. 2021). “Compared with non-bionic flying robots, biomimetic flapping-wing robot exhibit excellent flight performance and maneuverability and agility of biomimetic flapping-wing robot are better than that of the fixed-wing and rotary-wing flying robots” (Breuer 2019). For underwater salvage operations and deep-sea survey, underwater biomimetic robots which show travel efficiency and mobility are employed that surpass the outdated robot used in underwater robot (Dong et al. 2020).

Biomimetics in Superhydrophobicity

Several bioinspired biomimetic superhydrophobic surfaces recently developed were stimulated by natural organisms like wings of butterfly, leaves of lotus, which display excellent superhydrophobicity. Xu et al. (2016), report in-depth studies on “self-cleaning ability” due to superhydrophobicity shown by biostructures like duck feathers, skin of shark, and feet of gecko. Lotus’ flowers and leaves unfold and stayed clean even when emerging from mud and muddy waters. Water droplets acquire a spherical shape on lotus leaves that removes the dirt and impurities on the leaves when the surface is slightly sloped, showing self-cleaning function (Fig. 4). Due to this asset, it is widely replicated because of its latent advantages in multidisciplinary fields such as biomedical, environmental safety, aeronautics, polymer, textiles, rain wears, upholstery which never accumulate dirt, umbrella, etc. (Liu et al. 2014).

As Stegmaier et al. (2008) report, “self-cleaning textiles could be prepared via two approaches. One is addition of nanoscale coarseness on textile using silver nanoparticles, ZnO nanorods and carbon nanotubes, or by physical surface modification methods such as laser or plasma together with introduction of low surface

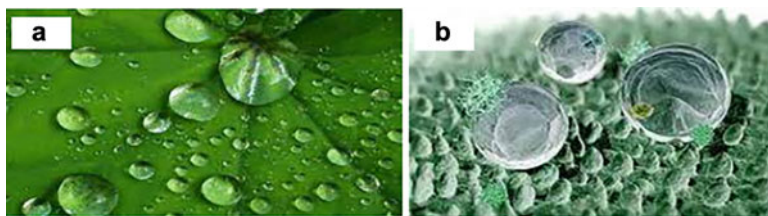


Fig. 4 “Water droplets on lotus leaves. (a) leaf surface microstructure formed by papillose -(small nipplelike projection, such as a protuberance on the skin, at the root of a hair or feather, or at the base of a developing tooth) epidermal cells covered with epicuticular wax tubules on the surface” (b) which create nanostructure” (Zhang et al. 2006)

energy materials which convey hydrophobicity.” Another method is “treatment of fabrics having micro- or nanostructure, like silicone coated on microfiber polyester fabric to introduce superhydrophobic polyester with self-cleaning properties” (Gao and McCarthy 2006).

Glass beads coated with paraffin were widely tested on the bases of lotus leaves for the property of hydrophobicity. “Recent addition to this includes the achievement of nano-microscale surface topography by fibre-attached nanoparticles that enhance surface roughness. Silicate and fluorocarbon nanoparticles when used serve the purpose commercially. This could be applied in fabrics, especially in cotton fabric using a homogeneous silica-copper hybrid nano-composite fabric to attain super hydrophobicity” (Liu et al. 2014).

Anticorrosion coatings bioinspired by lotus leaf were developed and are also replicated in paints industry, which could reduce the use of chemical detergents and costly labor in order to clean paints. As wetness damages concrete and metallic structures due to scaling caused by salt content and other chemical reactions which deteriorate the infrastructures, protective coatings inspired by biomimetics extend the life span of such structures due to repellency of water and self-cleaning action. “Many lotus-leaf-inspired biomimetic coatings like SiO_2 , polymethyl methacrylate – PMMA-ZnO nanocomposite, are UV-durable and thus would enhance the performance of materials and products used in outdoor conditions, as they can remain robust and stable to maintain their hydrophobicity with increasing UV exposure time” (Vazirinasab et al. 2018).

Inspired by the nonwetting phenomena of duck feathers, the microstructures of the duck feather showed a multiscale structure responsible for their superhydrophobic behavior. Chitosan, a biopolymer, when used as building blocks through a “novel surface solution precipitation (SSP) method and further modification with a silicone compound can achieve low surface energy.” Textiles treated with this material exhibit super water-resistant property, thus providing an automatic way to create hydrophobic surfaces on soft substrates using flexible material as building blocks. “Compared with lotus leaves, duck feathers are soft and flexible, and the water repellent characteristic of duck feathers is durable, thus it provides an ideal model for the fabrication of super hydrophobic surfaces on soft substrates” (Liu et al. 2008).

The lustrous feathers of *Anas platyrhynchos* drakes are due to “unusual arrangement of two-dimensional photonic crystals and further exhibit a superhydrophobic surface.” This property was recreated in a nanocomposite structure “fabricated by a typical top-down iterative size reduction method,” which showed enhanced hydrophobicity. As Khudiyev et al. (2014) reports, “It is prepared in 3 steps (1) fabrication of PC (polycarbonate) rod as a core component, which in turn is prepared using a hollow PC preform with an initial diameter of 30 mm, (2) A PVDF (polyvinylidene difluoride) polymer film is then wrapped around this rod until a total diameter of 12 mm is reached, and (3) a PC film is wrapped around the PC/PVDF core until the total diameter exceeds 20 mm (the complete architecture therefore has a final diameter ratio of 10: 2: 8 for PC: PVDF: PC). These 2D solid core photonic crystal fibers strongly resemble drake neck plumage in structure and fully polymeric material composition (Fig. 5) and can be produced in a wide array of colors by minor alterations during the size reduction process” (Khudiyev et al. 2014).

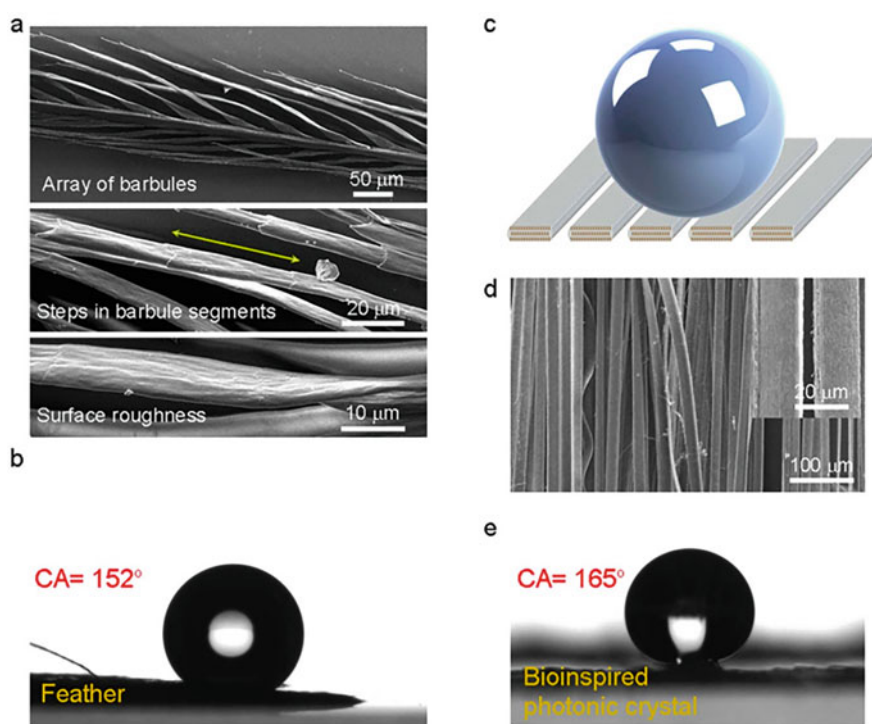


Fig. 5 Increased hydrophobicity in feather of drakes and feather-inspired photonic crystal structure. (a) Neck feathers exhibit super hydrophobicity due to the presence of structural hierarchy. (b) contact angle measurement of a single mallard feather. (c) enhanced intrinsic hydrophobicity of PVDF film by increasing surface roughness. (d) the nanoscale surface pattern observed on structures and the alignment of the fiber arrays both contribute to surface super hydrophobicity. (e) the water contact angle (CA) is measured to exceed 165° for this particular array. (Source: Khudiyev et al. 2014)

Biomimetics in Adhesives

Natural adhesion seen in biological organisms and natural materials has characteristics which the synthetic adhesive could not achieve. Examples include Velcro inspired by burdock plant, adhesive properties of insects which aid in climbing walls, and plants (“Ivy plant”) which show strong adhesion onto substrate-like buildings and those shown by barnacles (animals related to crustacea) with adhesion in “underwater habitat,” which help them to grip to ship hulls (Favi et al. 2014). Biobased adhesives have tremendous manufacturing industry applications due to their properties like biodegradability, renewability, and economical sustainability and do not pollute the environment.

Velcro (a popular brand of hook-and-loop binding), the finest example which works on imitating the principle of adhesion from a Burdock plant, is the “hook-and-loop fastener” developed by Georges de Mestral in the 1950s (Barthlott et al. 2017). Burdock is the “Velcro” of wild plants, whose seeds are inside the flower head, making its clinging characteristics an excellent mechanism for seed dispersal. Velcro, a good example of biomimetics, revolutionized multidisciplinary fields, which finds its application in routinely used things like buckle of shoes, curtain fasteners, fabric fastener, etc.

Another fine example is delicate adhesion shown by hairs in insects and lizards which aids them in climbing effortlessly without falling off. “Weak van der Waals interactions between the fine hairs on the adhering surface and the target surface paves way to adhesive property.” Geckos would stick to any surface, and this uncommon feature has created great curiosity to analyze the mechanism and fine structural characteristics of Geckos feet. “The gecko-feet have thousands of keratin fibers called setae which branches into hundreds of smaller and thinner fibers (Fig. 6). These micro and nano fibers ensure good adhesion with smooth and rough surfaces. This branch structure of the setae is important to adapt a large number of spatulas to rough surfaces and provide close contact with the substrate and induce intermolecular forces for the adhesion. Van der Waals forces and capillary forces play a dominant role in fibrillar adhesion” (Kim and Sitti 2006).

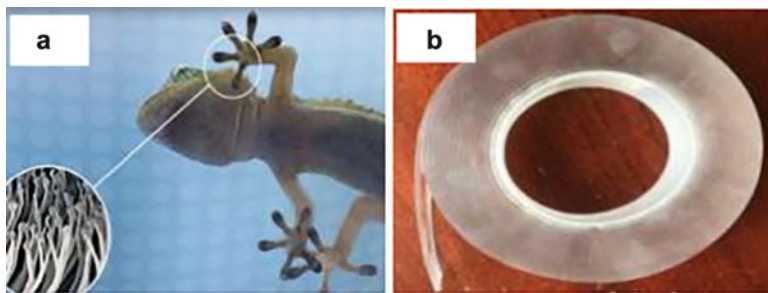


Fig. 6 (a) Gecko Toe-Seta arrangement which helps in adhesion; (b) carbon nanotube adhesive tape/gecko tape /nano tape

Scientists were enthused to imitate the gecko feet structure to make adhesion materials.

Synthetically made polymeric substances have been invented grounded on adhesive keratin fibers on geckos' feet (Shah and Sitti 2004). Apart from polymeric adhesion material synthesis, carbon nanotubes were also tried out, but firm adhesion is hard to achieve, and none were performed under water. "Studies indicated an improvement in the average shear strength of the adhesion with multi-wall carbon nanotubes" (Hsiao et al. 2003).

Study of adhesion properties shown by spiders and insects has also facilitated the development of synthetic adhesives, which led to evolve a designer foot for a "spider-inspired scaler robot," and further studies threw light on the factors which contributed to adherence property on different surfaces. These climbing robots find a wide range of application which would be used to reach unfathomable places for humans, "ranging from automatic cleaning systems for windows and exteriors of buildings, to the inspection of hazardous environments and autonomous vehicles for space applications."

"English ivy plant (*Hedera helix*, is an evergreen perennial) exhibits an excellent adhesion mechanism aided by secretion of a yellowish sticky substance when it climbs vertical surfaces." The superglue-like secretion, which comprises spherical nanoparticles, aids the plant to firmly grip onto surfaces as it creeps. Nanoparticles are composed of "arabino-galactan proteins," which aid in adhering strongly (Huang et al. 2016). Those nanoparticles displayed low-inherent viscosity in solution, and this gives a satisfactory wetting behavior to this adhesive. "Nanoparticles are thought to penetrate easily into any surface irregularities, further promoting intimate interactions with the substrate onto which the plant clings" (Chun 2016). Chun (2016), reports that "when the nanoparticles, pectin and calcium ions were combined, the bio adhesive characteristics were reproduced, further validating the adhesion mechanism."

Another entrant that shows excellent adhesion property is the algae named "*Prasiola linearis*," which is based on "non-specific protein folding and subsequent aggregation" which is the general property of polypeptides under certain conditions. This type of protein folding which leads to formation of "proteinaceous crossed β -sheet amyloid fibrils" is reported in neurodegenerative ailments like Alzheimer's disease. This "protein folding property" which provides mechanical strength could be extrapolated in new adhesives and other materials owing to their many valuable mechanical and self-assembly properties (Mostaert and Jarvis 2006).

Biomimetics is in close association with bionanotechnology, which has been described by Alvarado et al. as "the use of biological systems or its derivatives to manipulate the world at nanoscale" (Alvarado and Baudrit 2013). As Kaboorani and Riedl (2011), report, wise use of nanotechnology, besides imitating biological adhesive structures, is a breakthrough for the adhesive industry to create a new cohort of adhesives. Ramirez et al. (2019), show that "application of nanotechnology helped to design adequate alternative to old methods of wound closure, such as sutures and staples."

Biomimetics in Nanotechnology as Cross-Linking Polymers

Polymers are large molecules containing numerous small building blocks – or monomers – merged to form a chain-like structure which shows characteristics based on their component parts. Biopolymers are polymers that are produced by or derived from living organisms. Biomolecules like polysaccharides, proteins, and nucleic acids (NAs including DNA and RNA) are natural polymers which play key role in living systems owing to their intricate structures thus providing astonishing properties. Natural polymers provide a spur for biomimetics in creating artificial polymers which show explicit functions like “flexibility, non-toxicity, self-healing, adhesiveness, super hydrophobicity.” One way of growing these polymeric chains is through radical polymerization process, “where a free radical initiate chain growth by adding to a monomer unit, generating a new radical that in turns adds to monomer.”

Biomimetic polymers show wide range of applications like specific cell-focused drug delivery, modified biomaterials with enhanced tissue interaction like “transplants or prosthesis.” “The flexibility, ease of fabrication and low weight of polymers makes them suitable for extensive applications” (Camargo et al. 2009). Biomimetic synthetic polymers have the benefit of compounding chosen purposes with custom-made structure and stability at chemical and physical level. Translation of biological principles to synthetic polymers results in the fabrication of materials with greatly enhanced mechanical properties which finds its application in diverse fields.

In recent years, “the synthesis of biopolymers, particularly, the naturally available degradable polymers such as chitosan, hyaluronan, collagen and gelatin are used in the preparation of scaffolds, and these natural scaffolds are the total extracellular matrixes (ECMs) of decellularized tissues or organs” (Kohane and Langer 2008). “Biomimetic materials share characteristics with biomaterials in that they serve to restore natural function where the original material is absent or unable to perform properly.” “Biomaterials play a pivotal role as scaffolds to provide three-dimensional templates and synthetic extracellular-matrix environments for tissue regeneration” (Ma 2008). In the tissue-engineering field, biomimetic polymers can serve as scaffold materials via imitating the process seen in nature.

Due to properties like “biodegradability, biocompatibility and non-immunogenicity” of biopolymers like sodium alginate, chitosan, they are widely employed for medical and pharmaceutical applications (Motwani et al. 2008; Dang et al. 2012). Chitosan is “a derivative of chitin which contains N-acetyl-d-glucosamine residues and β -(1,4)-2-amino-2-deoxy-d-glucopyranose units.” Sodium alginate is a polysaccharide made from brown sea weed which consists of repeating units of α -l-guluronic acid and (1,4)- β -D – mannuronic acid. These two biopolymers have high end applications in biomimetic polymer synthesis. “Wound dressings derived from natural polymers such as Alginate and chitosan-based wound dressings have plentiful advantages as they can take up several forms such as sponges, foams, nanofibers, films, and hydrogels” (Huang and Fu 2010; Mndlovu et al. 2019).

However, natural polymers are usually insufficient due to their disapproving mechanical characteristics. Synthetic polymers, when used in combination with

natural ones, seemed to show “enhanced physical properties, degradation patterns, and overall antimicrobial properties” (Almeida et al. 2011). Recently, using the “solvent casting technique,” Balu et al. (2020), fabricated “biomimetic nanocomposite scaffolds which contain chitosan–sodium alginate blended with three different TiO_2 concentrations (used as fillers).” The nanobiocomposite showed decreased cytotoxicity and better antibacterial activity which showed positive results. Hybrid nanobiocomposites, made from inorganic metal oxide nanoparticles as fillers with large surface area, interact effectively with polymer chains at molecular level. As per existing reports, “nanostructured Au, SiO_2 , CdS, ZnO, TiO_2 , etc. based bionanocomposites are widely used in biomedical applications” (Moura et al. 2016). Biocompatible and bioactive substances from biological sources fulfill various functions that are critical for the maintenance of life.

Hydrogel and Lipophilic Polymers

Wisely created biomimetic polymers, for biomedical use and tissue engineering or as implants, show successful interaction with “cells/multiple cells or even whole tissues.” “The polymers used can be divided into two major classes based on their physicochemical properties; hydrogels- composed of hydrophilic polymers which swell in water, from solid lipophilic materials which demonstrate little water uptake when brought into an aqueous environment” (Langer and Peppas 2003).

Hydrogels allow increased oxygen, drugs, and nutrients dispersion rates (Lee and Mooney 2001) and can be administered with or without cells, allowing for a less invasive implantation. Their fluidity allows ease of shape flexibility and stiffens via gelation (Drury and Mooney 2003). Cross-linking makes hydrogels acquire mechanical firmness. “Cross linking either physically or chemically gives hydrogel a 3D network structure, making it insoluble. This insoluble cross-linked structure allows effective immobilization and release of active agents and biomolecules. Hydrogels appear similar to natural soft tissues because of their high-water content” (Hubbell 1995).

Hydrogel polymers used in tissue engineering range from “naturally derived to synthetic materials” which show better biocompatibility and diminished cytotoxicity. “Alginate, gelatin, agarose, fibrin, chitosan are examples of naturally derived polymers, whereas poly(ethyleneglycol) (PEG), oligo(poly(ethylene glycol) fumarate) (OPF), poly (acrylic acid) (PAA) derivatives, and poly(vinyl alcohol) (PVA) represent synthetic polymeric materials” (Drotleff et al. 2004).

Alginate is a “linear polysaccharide copolymer of (1–4)- linked beta-D-mannuronic acid and α -L-guluronic acid,” due to its features like reduced toxicity and ease of accessibility, and is altered with peptides with free carboxylic acids on the polymer backbone under minor gelation settings. “Alginate gels can be cross-linked using divalent cations (Ca^{2+} , Ba^{2+} , or Sr^{2+}) or by covalent chemical cross-linking techniques” (Lee and Mooney 2012). Arginylglycylaspartic acid (RGD), the “peptide motif responsible for adherence of cell to the extracellular matrix (ECM), is exhibited in most organisms. Cell adhesion proteins called integrins – “principal receptors used by animal

cells to bind to the extracellular matrix” – function as “transmembrane linkers, fix to this sequence, which is found within many matrix proteins, including fibronectin, fibrinogen, vitronectin, osteopontin, and several other adhesive extracellular matrix proteins” (Plow et al. 2000). The unearthing of RGD and illumination on how it interacts to integrins paved way for the invention of advanced therapeutics and diagnostics. “The peptide itself is used ubiquitously in bioengineering” (Alipour et al. 2020). “Cell-alginate interactions is enhanced by covalent linking to integrin binding peptide sequence RGD or its derivatives to the polymer backbone. The free carboxylic groups of the latter are activated using EDC/NHS (ethyl(dimethylaminopropyl)carbodiimide / N-hydroxysuccinimide.) and reacted with the terminal NH₂-group of the peptide” (Rowley and Mooney 2002) (Fig. 7a).

Chitosan is an excellent polymer used as a suitable substrate in creating biomimetic polymers, due to its organizational resemblance to glycosaminoglycans found in native tissue and presence of easily alterable free amino groups in the polymer backbone (Drury and Mooney 2003). Wang et al. “covalently bound WGA (Wheat

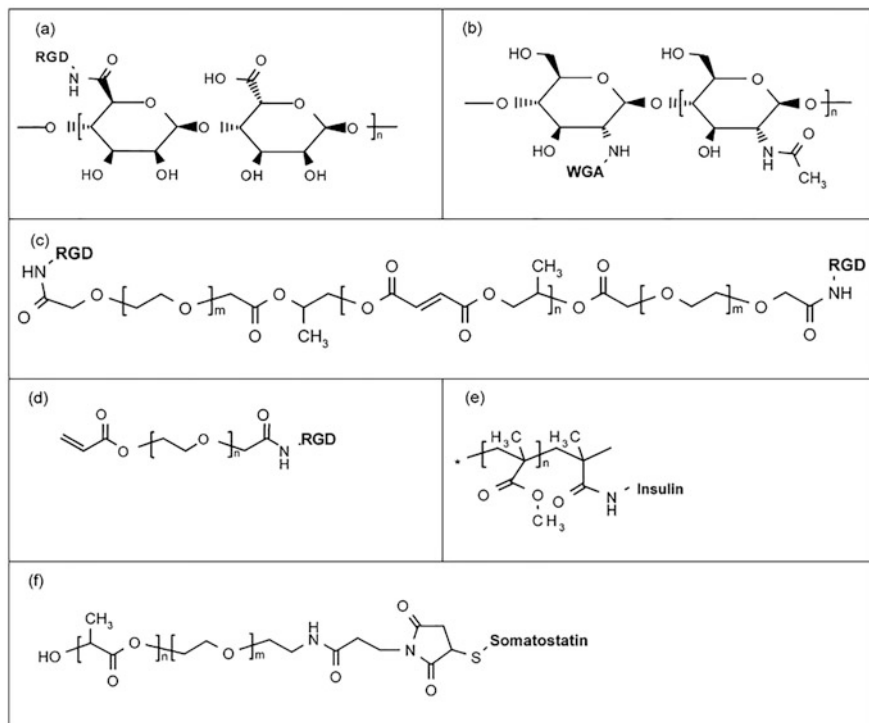


Fig. 7 An illustration of examples of biomimetic polymers. “Materials derived from **natural** hydrogel type polymers: (a) alginate-RGD; (b) chitosan-WGA. Materials derived from **synthetic** hydrogel type polymers: (c) PPF-PEG-RGD; (d) (PEG-RGD. Materials derived from **lipophilic** polymers: (e) PMMA-Insulin; (f) PEG-PLA-Somatostatin” (Image Source: Drotleff et al. 2004, European Journal of Pharmaceutics and Biopharmaceutics 58 (2004) 385–407)

germ agglutinin), a lectin molecule, to chitosan to enhance cell–biomaterial interactions by first activating WGA using EDC and afterwards reacting these products with the amine groups of chitosan to form stable amide linkages” (Wang et al. 2003) (Fig. 7b).

A commonly employed synthetic polymer in the biomedical field is Poly ethylene glycols (PEG), owing to nontoxic and nonadhesive property to protein which results in excellent products. “PEG can be processed into a hydrogel by modifying with acrylates or methacrylates, which are sensitive to photo-cross-linking” (Drury and Mooney 2003). To enable selective cell attachment, PEG is attached to RGD-sequences (Drumheller and Hubbell 1994; Burdick and Anseth 2002) (Fig. 7d).

Another synthetic hydrogel-forming material for drug conveyance applications is “an amphiphilic triblock copolymer derived from a low molecular weight poly (propylene fumarate) (PPF) with two terminal PEG units.” It results in a product being minimally invasive, due to its property like being decomposable due to hydrolytically cleavable ester groups in the polymer backbone and thermo-reversible gelation. Jo et al. synthesized “a triblock copolymer consisting of two terminal carboxymethyl PEG units and one PPF block in the middle of the copolymer” (Jo et al. 2000). The end free carboxylic group which is unrestricted allows for conversion to succinimidyl esters using N-Hydroxysuccinimide/ dicyclohexyl carbodiimide (NHS/DCC) chemistry resulting in polymers, easily modified with RGD sequences (Fig. 7c).

Current research shows that in addition to hydrogels, lipophilic water-insoluble polymers are also employed to make biomimetic polymers. Degradable materials are typically used for biomedical applications during gradual reabsorption of the material thereby replacing the defect in living tissues. Nondegradable materials have been explored, to accomplish increased biocompatibility and tissue incorporation of medical implants. Many nondegradable materials like polystyrene (PS) or polyacrylate have furthermore been modified to yield biomimetic materials. Polystyrene (PS) offers a good model system for lipophilic surfaces. Park et al. synthesized a “sugar-bearing PS derivative with RGD grafted to the polymer backbone using carbodiimide chemistry to investigate the changes in the behavior of hepatocytes on these modified polymer surfaces” (Park 2002).

Poly (methyl methacrylate) (PMMA) is another material which can easily be altered by the hydrolysis of methyl ester groups in a basic environment. PMMA surfaces can be bound with peptides through “subsequent reaction of the obtained acid residues with amine groups of peptides using EDC chemistry” (Ito et al. 1996). A substitute constitutes the fastening of the bioactive molecule to an acrylate anchor and grafts it to the PMMA backbone using UV-irradiation. Schaffner et al., for example, used this method to covalently link insulin to PMMA surfaces (Schaffner et al. 1999) (Fig. 7e).

PLGA – (Poly (lactic-co-glycolic acid) and PLA – poly (lactic acid) materials are persistently used for biomedical applications because of their exceptional biocompatibility to form firm scaffolds for cell cultivation (Quirk et al. 2001). Free carboxyl group at the terminal of PLGA chains aids in modification of the polymer. In one type of PLGA modification, “a galactose derivative was bound directly or via a PEG

spacer to the acidic end of the molecule” (Yoon et al. 2002). “Using NHS/DCC chemistry, an amine containing galactose derivative or PEG diamine was tethered to the polymer. Lactobionic acid was then grafted to the remaining free amine group of PEG using carbodiimide chemistry. An alternating block copolymer of lactic acid and lysine was used to provide free reactive amine groups in the polymer backbone.” RGD peptides were then covalently attached to the resulting free amine groups (Cook et al. 1997).

Tessmar et al. (2003) created a new group of PLA derivatives. “To reduce uncontrolled protein adsorption to the lipophilic PLA, a diblock copolymer with hydrophilic PEG was synthesized, starting from PEG and D, L-lactide in the presence of stannous 2-ethylhexanoate” (Tessmar et al. 2002). The amine group at the terminal end of PEG chain showcases a modification site. By employing linkers like L-tartaric acid or succinic acid, the polymer was activated for protein binding by converting amine group to a reactive carboxylic group. “Alternatively, a thiolreactive group was introduced via β -alanin and maleic acid anhydride resulting in a thiol reactive maleinimide. Insulin, as an amine containing protein, and somatostatin, a substance with a cleavable disulfide bridge, were shown to attach to these activated polymers” (Tessmar et al. 2003; Drotleff et al. 2004). For wide application in tissue engineering, Hacker et al. developed a method for producing scaffold which maintains its binding capacity resulting in forming cell carriers which are easily modified with proteins (Hacker et al. 2003).

Thus, as discussed previously there are enormous examples of polymeric materials which act as hydrogel and lipophilic polymers used in tissue engineering and serve greater purpose in the biomedical field. Specific type can be chosen based on the purpose.

Polymeric Synthetic Pathogens

Another contribution in the field of biomimetic polymer is creation of polymeric synthetic pathogens where “the features of pathogens, in particular viruses, which possess diverse geometry and surface properties are exploited.” Viral properties are simulated in “bio-inspired non-viral polymeric systems for high efficiency drug/gene delivery and other therapeutic applications.” Polymeric nanoparticles which embed DNA have been made with variance in geometry to biomimic the structure of natural viruses. The “constructs are composed of a cationic co-polymer and anionic DNA that, through bottom-up self-assembly in solvent with varying polarity, can form nanocomplexes” (Jiang et al. 2013). These polymeric nanostructures specifically target cells or tissues which can possibly modify target cells – immune system cells and stem cells – and induce them to act based on information encoded via nucleic acids incorporated in those nanostructures. This method ensures safety without being infectious. Better immunization against pathogenic diseases is achieved via this method. “Lipid-coated polymeric particles which display antigen on the surface” are one such approach of creating polymeric synthetic pathogen (Hanson et al. 2014). This approach permits “individual tuning of the core-shell structure.” To

mimic pathogens for better antigen presentation to stimulate immune cells for antibody production, better ways of tuning the properties of the polymeric particle are needed. Ajithkumar and Pramod (2018), established “a virus system with a core-shell structure for targeting tumor tissues with a simple and predictable self-assembly technology.” They incorporated doxorubicin – a specific anticancer drug – and DNA into the core of artificial virus and a folate-bound albumin as outer layer. As folate receptors are persistently over-expressed in cancer cells and normal cells show limited expression, as per Lu and Low (2002) this allows specific adherence of artificial viruses to cancer/tumor or any type of abnormal cells. As Zheng et al. report, “The outermost layer of the artificial virus is a polypeptide complex of poly (L-lysine) and poly (G/L-glutamic acid), which dissolves in acidic pH. Hence, when an artificial virus is introduced in vivo, it reaches the tumor tissue, the complex dissolves and the drug releases ensuring targeted drug delivery” (Zheng et al. 2021).

Biocomposites

Ebrahimi and coworkers 2019, reported “biomimetics in the field of tissue engineering, which focuses on mimicking the biological process during biomaterials synthesis to produce materials similar to natural tissues” (Ebrahimi 2019). These materials have wide range of application in the biomedical field. From biomimetic point of view, “natural tissues are composite materials with a structural unit based at the nanoscale.” Attempts on development of new materials should be based on the complex design and characteristics as well as its scale at micro- or nanolevel. “An exceptional example is 3D printing of organs.” Abouzeid et al. (2018) reported “the preparation of bioactive scaffolds for bone tissue engineering by combining 3D printing and a biomimetic mineralization process.”

3D printing of bones aided by biomimetics is studied by Lopes and coworkers (Lopes et al. 2018). The “biomimetic mineralization” of bones encompasses development of hydroxyapatite (HAp) by the presence of calcium ions and phosphate ions in replicated body fluid on the scaffold surface. This allows uniform dispersion of HAp in the scaffold. “This modification is analogous to chemical structure and material properties of natural bone minerals and has got higher biological activity and promotes cells proliferation and growth” (Lopes et al. 2018). This method’s demonstrations enhanced mechanical properties to encounter the requirements of bone tissue repair, while “scaffolds without biomimetic mineralization are soft and prone to collapse.”

As per Hopkins et al. (2015), “due to complexity of its structure and function, the central nervous system (CNS) is challenging to mimic.” There is increased occurrence of neurological diseases. As neurons are cells that cannot be regenerated under any conditions, a ground-breaking approach toward construction of neurons and auxiliary cells would be highly valuable to mankind. A biomimetic approach toward spinal cord shows that, “the types of cells and their arrangement has a highly controlled spatial distribution which is the key to mimic the spinal cord architecture.” Recently, Joung et al. (2018), “fabricated a heterogeneous bioinspired spinal

cord by multi-material 3D bioprinting in which clusters of spinal Neuronal Progenitor Cells (sNPCs) and Oligodendrocyte Progenitor Cells (OPCs) can be accurately positioned in different parts of the scaffold during assembly” (Fig. 8). These “bioinspired spinal cords successfully showed differentiation of functionally mature neurons during in vitro culture, which allows for further differentiation of neuronal

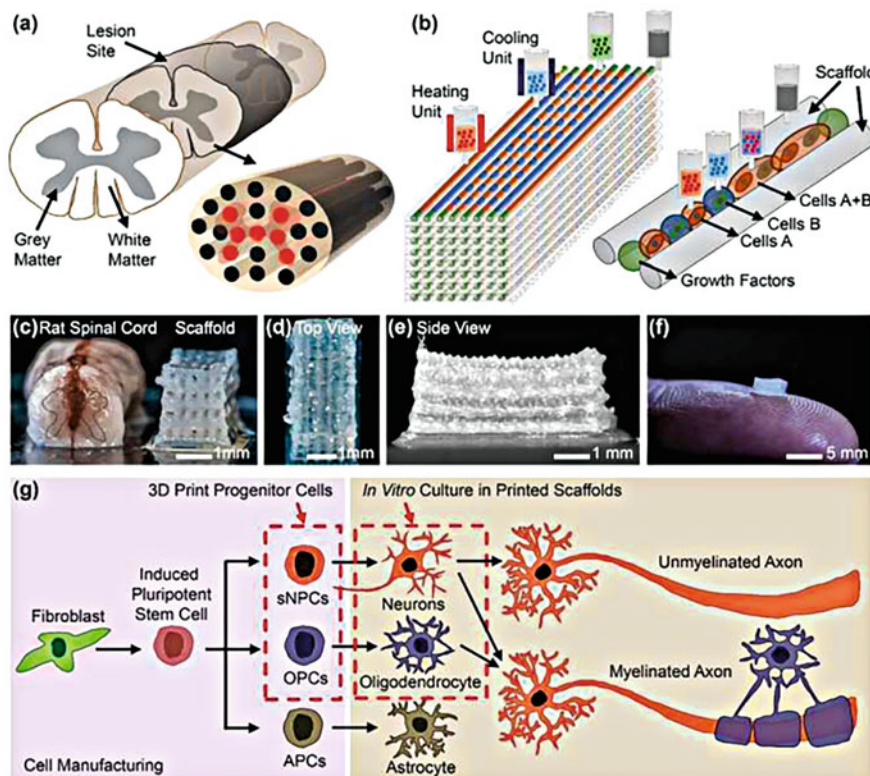


Image Source - <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7319181>

Fig. 8 “Experimental strategies for 3D bioprinting spinal cord tissue.” “(a) Schematic of the spinal cord illustrating grey matter and white matter boundaries and a design for a 3D bioprinted multichannel scaffold for modeling the spinal cord. (b) Schematic overview of the 3D bioprinting process. Biocompatible bioinks are extruded at specific temperatures (37 °C or 4 °C, depending on the bioink) in a layer-by-layer process. The scaffold ink is structurally supportive and can be made with a biocompatible material. (c) Comparison of a transected rat spinal cord and the design principle for scaffolds consisting of multiple, continuous channels. The number of channels can be scaled according to the size of the scaffold needed. (d) Top view image of scaffold channels demonstrates a printing resolution of ~150 μm. Channels are continuous throughout the scaffold, allowing for axonal extension. (e) Side view of a 5 mm long scaffold. (f) A 2 × 2 × 5 mm₃ sized scaffold on top of a finger shows the scale of a scaffold. (g) Schematic of induced pluripotent stem cell (iPSC) reprogramming and differentiation into spinal neuronal progenitor cells (sNPCs) or oligodendrocyte progenitor cells (OPCs). These progenitor cells are 3D bioprinted into the scaffold and cultured in vitro.” (Source: Joung et al. 2018)

progenitor cells into neurons projecting axons and oligodendrocytes that myelinate the axons, respectively. This biomimetic approach provides a novel technique for treating neurological diseases which aids to prepare scaffolds with better bioactivity for mimicking the central nervous systems” (Joung et al. 2018).

Dentistry

In dentistry, application of biomimetics includes repair of dentition by inculcating possible ways of regenerating and replacing affected dental tissues by biomimicking natural tooth in properties and morphology. Curative dentistry offers a varied variety of applications like reestablishing defects in tooth by polymeric biomimetic peptides to attain remineralization (Bazos and Magne 2011). As per Tirlet et al. (2014), “adhesive restorative materials have demonstrated tooth morphology and aesthetics mimicking natural teeth. Biomimetic dental implant coatings of calcium phosphate and hydroxyapatite have been investigated to improve osseointegration of dental implants to achieve therapeutic benefits (Galler et al. 2010). “The tissue-engineering approaches resulted in regeneration of lost or damaged dental tissues mimicking their natural counterpart” (Zafar et al. 2020). This therapeutic method endeavors preservation of intact tooth structure and restores the role of natural teeth. For example, “Biomimetic endodontic regeneration includes the formation of dentin barrier by pulp-capping agents, root formation during apex genesis and apexification, apical healing by root-end fillings, and pulp regeneration by cell-homing strategies” (Kaushik et al. 2016).

Advantages and Limitations of Biomimetic Materials

The science of biomimetics provides opportunity to learn various strategies for survival and energy efficiency showed by different life forms and apply them to bring sustainable solutions to the challenges faced by mankind in innumerable fields. Apart from imitating biological materials or services, biomimetics requires thorough study of organisms and where they inhabit along with how they function. Without further acquiring knowledge about such works, the utility of mechanisms cannot be accurately understood.

Biomimetics is based on the properties that ensure some integral advantages observed in the biological world. It helps create products and processes that are sustainable, cost effective, energy efficient, and high performance and that redefine and eliminate waste. As Bar-Cohen (2006), reports, “assistance from the study of biomimetics can be seen in many applications, including stronger fibre, multi-functional materials, self-cleaning super hydrophobic biomaterials, improved drugs, superior robots, sustainable architecture, strong adhesives, restorative dentistry, bioengineered spinal cord, energy harnessing structures which run on sun’s energy.” In spite of all these advantages, the science of biomimetics offers many drawbacks and limitations at application level. As Shimomura (2010), states, “the combination of newly revealed materials with biomimetics research will be a key to

understand their applications and limitations.” The structural and purposeful usages of new biomimetic products must be perceived together with positive and negative characteristics study.

A challenging segment in biomimetics is the identification and selection of the initial source of biological information which serves as solution for a scientific problem. This primary source of bioinspiration must not be highly specific, comprising several organizational levels of the biological domain which allow a vast variety of biological phenomena covered, and must not be too complex, to facilitate the process of understanding for those investigators without a biology background.

Identification of functions of organisms along with their various adaptive mechanisms in response to environmental factors based on energy optimization is an ongoing field of research which forms the basis of biomimetics. The supreme encounter faced by biomimetics is to elucidate the association of fine structures in biological sources and its relationship with the organism and its habitat, if they are not wholly studied. “The designation ‘nature made’ does not provide intrinsic superiority.” Shimomura (2010), showed that “finding substantial examples through the integration of biology, natural history, and materials science is the next step in biomimetic research.”

Adhesion characteristics of various biopolymers are a matter of great concern as they have wide range of applications in various fields. Even though strength of adhesion property is better in synthetic/nonsynthetic adhesives, in the biomedical field they have limitations like low absorption rate, high toxicity unsafe for life, and less adhesiveness on wet materials. Most of the adhesiveness shown by natural materials is due to their nanostructures, and hence research has to be focused on new adhesives based on nanostructural level. However, to develop an appropriate adhesive for different surface types is still not possible.

Another limitation in biomimetic polymer design is the constraints in manufacturing technology currently practiced. In nature, organization of building blocks into specific patterns or geometry occurs with incredible integration. But to recreate or add the property in a bioinspired product is highly complex, and such intricacies need to be standardized primarily. In addition, some of the high-performance products synthesized in laboratory occur with restricted size and quantity and are minimal, and the characteristics are analyzed without following uniform standards which draw back further work. Time and cost investment in the process also adds to the limitation when large-scale production is concerned. As per Wang et al. (2020), “the inherent efficiency of biological materials is difficult to duplicate, as the microstructure involves engineering complex architectural details that are beyond the capability of most current nanotechnologies.” Therefore, in summary, such bioinspired biomimetics cannot be replicated at an industrial scale and technological mapping of the process is over whelming at the moment.

In the forthcoming times, several biomimetic polymeric materials, which can be used as implants which can be applied clinically, will be developed regarding the current scenario of research works. In dentistry, “the reinforcement and completion of the tooth structure by biological regeneration will be evident in few years” as per Zafar et al. (2020). But due to the complexity of tooth structure, limitations and challenges for application clinically need to be overcome.

Knowledge acquisition on the various natural phenomena and detailing of natural materials may not always solve difficulties or challenges faced during biomimetic polymer production; nevertheless, possibilities and potential of further research work are tremendous. As Reed et al. (2009) cite, “Today’s biological curiosity can become the object of tomorrow’s biomimicry.”

Future Prospects

Currently, different types of biomimetic polymeric materials are widely employed for variety of functions. However, as new mechanisms for the production of nature-inspired polymeric materials are formulated, progression of biological bodies and the mechanism of how they evolve over a time period also need to be distinguished. As the scientific community’s awareness of how various life processes occur, various biomaterials’ involvement and their hierarchy in structure and better knowledge about working code come to light, and interdisciplinary research can be carried out to imitate their technology for innovative products. Biocompatibility of presently used polymeric materials also needs improvement, and hence, extensive research needs to be carried out.

Biomimetic polymers offer great promise to actively modulate biological systems. Numerous challenges need to be overcome in the area. The knowledge sparsity about structure and functioning of the biological system, which is the source of biomimicry, is the biggest challenge in this field of biomimetic polymers. Strong perception of principles ruling biological organisms and phenomena of various life processes is a significant aspect in biomimicry. The various biomimetic materials discussed in this chapter are useful tools to explore and illuminate biological principles. The competences of biomaterials beneficial for use in biomedical research develop drastically during the period when exploration can move toward understanding of how such materials interact with the biological system both locally and systemically.

Recently, biomimetic polymers in the biomedical field, specifically the immune system, are a focus for manipulation. Using polymeric materials to target precise immune system constituents in combination with drug delivery has the latency to pointedly increase the capability of cancer immunotherapies to better target tumors. This would be revolutionary as it expands the disease resistance. It is anticipated that exceptional biomimetic models created based on the knowledge of different biological bodies will enhance the life and application of polymeric biomaterials. Presently, the biomimetic process is moving at a slow pace in revealing the full potential of biomimicry.

Conclusion

Nature has shown evolution and augmentation of different kinds of biomaterials with exceptional characteristics. Deep thoughtful investigation into the mechanism behind this creation aids to recreate useful products which serve human beings.

Biomimetics and fabrication of nature-inspired polymers has been the driving force for many innovative ideas and research for years. The commercial applications of biomimetics are widespread including devising new nanomaterials, devices, and processes. With the fast progress of research and knowledge built up, biomimetics accelerates the biomedical field forward to introduce new biomaterials which mimic nature. Imitating the characteristics will likely lead to innovative products; nevertheless, it has boundaries which need to be overcome, which brings greater impact in biomedical field which would solve many snags at the application level. With the simple idea of imitating nature, now biomimetics has touched many sophisticated and delicate areas of science and technology with amazing results. Advancements in biomimetics and nature-inspired biopolymers hold the key to more sustainable technological developments.

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Abstract

Because of its biocompatibility, biodegradability, its lack of toxicity, antigenicity and high ligand-binding capacity, albumin is widely used in the cosmetic applications. Albumin have improved functional properties and they are used for the

R. Rajakumari

Department of Pharmaceutics, Krupanidhi College of Pharmacy, Bengaluru, Karnataka, India

e-mail: rajimpharm@gmail.com

S. Thomas (✉)

Department of Pharmaceutics, Mahatma Gandhi University, Kottayam, India

School of Energy Materials, Mahatma Gandhi University, Athirampuzha, Kerala, India

N. Kalarikkal (✉)

Department of Pharmaceutics, Mahatma Gandhi University, Kottayam, India

School of Pure and Applied Physics, Mahatma Gandhi University, Athirampuzha, Kerala, India

e-mail: nkkalarikkal@mgu.ac.in

delivery of cosmetic ingredients. The present work discusses the cosmetic applications of biopolymeric materials which include the interaction of materials with the skin and important characteristics of topical delivery of bioactives, safety measures of albumin materials in cosmetics, various design formulations of albumin, and the particular applications in the field of cosmetics. Albumin plays a critical role in the health as it is one of the major proteins in the living organisms.

Keywords

Albumin · Cosmetics · Application · Skin · Proteins · Biodegradability

Introduction

The use of cosmetics is not a modern part of application because ancient women used Kohl to darken the eyelashes and Cleopatra used milk to whiten and soften her skin. Now in today's world, cosmetics are billion dollar industry which uses a wide range of materials like minerals, polymers, emulsifiers, preservatives, color, flavors, thickeners, and pH stabilizers. Use of biopolymers in cosmetics is widely classified into proteins and polysaccharides. Use of albumin in cosmetics became more advantageous because they are biologically derived, biocompatible, biodegradable, and eco-friendly. Proteins from animals, fungi, and algae are widely used as source of protein. In recent times, plant-derived proteins are also used in cosmetics. The safety and effectiveness of proteins/peptides in cosmetic formulations is well established for skin and hair care. In the moisturizer, peptides are known to improve the appearance of facial skin. The polysaccharides are inexpensive and readily available because of their natural sources, and they have been used in cosmetics for a long time. The polysaccharides widely used in various cosmetic applications are cellulose, xanthan gum, hyaluronic acid, chondroitin sulfate, guar gum, chitin, dextrans, pectin, carrageenan, agar, and alginates (Luo and Wang 2016; Mota et al. 2020a).

A cosmetic material is defined as a constituent or preparation proposed for placement in contact with any external part of the human body, including the skin, mouth, and teeth. We use cosmetics to protect, cleanse, perfume, and change the appearance of our skin or to alter its odors. Various biopolymers such as proteins and polysaccharides were used to increase the aesthetics of the product and to improve the effect on the body. These are used as emulsifiers, thickeners, whitening agents, antiwrinkle agents, etc. (Mota et al. 2020b). Usually, cosmetics contain a mixture of water, emulsifier, preservative, thickener, and stabilizers of color, fragrance, and pH. Apart from these ingredients, some active formulations called cosmeceuticals that impact the natural function are also included in the products to give some pharmaceutical effect. Common classes of ingredients used in cosmetic formulations are specified in Table 1 (Augustine et al. 2013).

Cosmeceuticals is the integration of cosmetics along with the pharmaceutical industry. They are selected by real effectiveness, and they contain active ingredients

Table 1 Class of ingredients used in cosmetics

Function	Common classes of ingredients
To promote the emulsion formation	Emulsifiers
To change the consistency of product	Thickeners
To increase the hydration	Moisturizers
To decrease the surface tension of the product	Surfactant
To give enjoyable aroma to the product	Fragrances
To provide an pleasing color to the product	Colors
To regulate the pH of the product	pH stabilizers

Table 2 Class of ingredients used in cosmeceuticals

Function	Common classes of ingredients
To maintain a youthful appearance	Antiaging agents
To prevent the lines and wrinkles	Antiwrinkle agents
To eliminate localized hyperpigmentation and lighten the general skin tone	Whitening agents

at high concentration with their prevention and inherent treatment features. They are manufactured with a greater scientific evidence than the normal cosmetics and hold a lower therapeutic value than the pharmacological product. Like cosmetics, cosmeceuticals are topically applied but they contain ingredients that influence the biological function of the skin. Cosmeceuticals deliver the necessary nutrients for improving the appearance for healthy skin. Cosmeceuticals improve the skin tone, radiance, and texture, by reducing wrinkles which forms the increased growth section of the personal care industry. The most popular cosmeceuticals are given in the Table 2 (Pandey et al. 2022; Patravale and Mandawgade 2008).

Albumin is a blood protein that is mostly produced in hepatocytes and it acts as a reservoir for the transport of many substances such as nutrients, hormones, metals, and toxins. They are produced in the hepatocytes; the molecular weight of albumin is 66.5 kDa and the half-life is 19 days. The albumin is available in different variants such as bovine serum albumin, ovalbumin, and human serum albumin (HSA). HSA plays an important part in the drug delivery system because of its biodegradable nature. The ovalbumin is easily available, low cost, and bioavailable, and they are used in food, cosmetics, and drug delivery applications. This albumin can be able to associate through covalent or noncovalent attachment, covalent conjugation, and ligand mediated interactions with the bioactives or encapsulation of the bioactives (Foulke 1992). The traditional method uses the albumin in egg white that have been widely used as a wrinkle remover for centuries, and when the egg white is applied to the skin, it provides tightening of the skin and smoothens the wrinkles. After the egg white is applied to the skin, the tightening effect of the skin is produced within 5 to 10 mins, and albumin gets dried on the skin. Usually after applying the egg masks the face has to be kept without any movements, otherwise the mask would crack and

the tightness of the skin gets lost. Most of the skins will get benefitted from the egg pack and it is one of the age-old cheapest form of beautifying treatment method. The method involved is to separate the white from the yolk of the egg and beat the white till it gets fluffy. Lemon juice is added to the beaten white which is then applied to the clean face, and it should remain on the face for about 20 min. Then the pack is removed with warm water which is followed by washing with cold water and finally a piece of ice is applied over the skin. These egg mask or egg pack treatments include astringent and cold water or ice to sustain the tightness of the skin (Gadomska and Katarzyna Musiał 2021; Yusuf and Fatmawaty 2017).

Another example is the use of bovine serum albumin (BSA) as a wrinkle smoothening agent. In this method, they have formulated the BSA with the film forming agents, plasticizers, and preservatives. The film forming agents such as sodium silicates and carboxymethyl cellulose help deliver the uniform film with a matt finishing so that it is less visible. The plasticizers such as glycerine or propylene glycol increase the flexibility of the film so that it prevents from scaling, cracks, and flakes. These ingredients together improve the smoothening of the wrinkles which simultaneously increases the visual effectiveness of the skin (Zhu et al. 2008).

In a study, they made an albumin solution and applied on the facial skin and found a tightening sensation on their skin because the solution dries within few minutes, and reduction of wrinkles and smoothing effect was observed within 3–8 mins. They have performed a microscopic examination to visualize the facial skin after application of albumin solution and observed that even the finest wrinkles became invisible. The expression lines around the eyes and mouth are not altered much but when larger amounts are applied there are reduction in the fine lines. When larger amounts are applied it reduces minor wrinkles and also it leaves a transparent film which later turned into scale flakes. By applying it repeatedly leads to peeling after it dried off and the effect is about 2–6 h depend on its facial motion. The time extends for the person whose face showing little emotion, inexpressive and poker faced. When the face gets dried, it is remoistened with a few drops of water to make it restored. With the wetting of water, it reduces the fine wrinkles even after 8 h, and also it is said that completely washing the face with soap and water will completely eradicate the smoothening effect. This anti-wrinkling effect is not cumulative with the repeated dosage daily and there is improvement from the first to last day of use. After the applied solution gets dried, applying the facial creams did not alter the effect and also no adverse effects and side effects were observed. This chapter will provide a highlight on the structure, molecular association of albumin, and role of albumin in the formulation design for the cosmetic applications (Li et al. 2016; Hu 2011).

Albumin-Based Tissue Scaffolds

In this method they have used a new process in which it is converted into an albumin polymer from the liquid albumin solution and then into a tissue scaffold by freeze drying-based molding method. The yield is about 75% with respect to

volume and for single 500 ml of blood, the yield of albumin tissue scaffold would be 105 ml. So, the availability of this material will be adequate for the tissue engineering applications, and it showed an excellent biocompatibility. Normally, the albumin is used as an additive in the tissue culture medium because the cell responses are associated with this protein. Moreover, albumin is the major protein present in the animal blood and the plasma albumin binds with the fatty acids, nutrients, steroids, and metabolites in the circulation. For maintaining pH, osmotic stress, and reducing the power of human blood, the plasma albumin plays a major role. In this method, they have mentioned that albumin-based tissue scaffold is considered as a useful substrate because it plays a structural support in the cell and tissue engineering applications (Ong et al. 2019). The physical and mechanical properties revealed that the prepared albumin scaffolds were similar to that of the collagen tissue scaffolds. The albumin tissue scaffolds and the collagen tissue scaffolds have exceptional water binding capacity. In addition, they also have good cell binding characteristics which support cell growth because they are peptide-based biomaterials. In this study, they have found that the albumin tissue scaffold didn't change in the dimensions even after 28 days of MSC culture. The albumin tissue scaffolds are difficult to degrade by MSCs because the degradation behavior of cross-linked albumin is very different from the other biopolymers. Because of this unique characteristics, the albumin will decrease the complexity by avoiding the interactions between the cell and scaffolds. This albumin tissue scaffold is a good substrate for tissue culture and tissue engineering applications (Li et al. 2014) (Fig. 1).

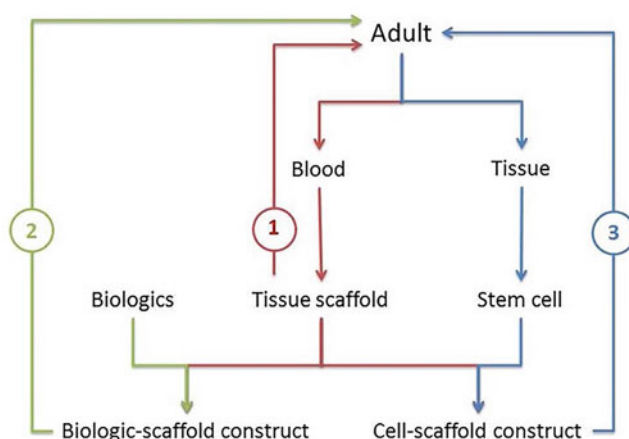


Fig. 1 Albumin tissue scaffold in tissue engineering. Blood and tissue are collected from an adult and an albumin tissue scaffold is developed, seeded with stem cell and with biologics to form biologic scaffold composites. Albumin tissue scaffold forms an artificial tissue with a living cell scaffold

Albumin Protein from Rice Bran

The most important crop is rice in most parts of Asia and it gives more than 20% of energy source and it is the very significant crop in nutrition. During the polishing process of the rice, the by-product obtained from the manufacturing process is said to be “Rice Bran” which is about 10.5% of whole grain. This rice bran is rich in nutrition with high value of protein when compared to other parts of grain. Rice bran consists of vitamin B, Vitamin E, minerals, dietary fiber, and rice bran oil which contains unsaturated fatty acids. The protein in the rice bran are albumin, globulin, prolamin, and gluten which nourish the body with protein, peptides, and essential amino acids. Moreover, it also helps to build immunity and is an important component in food and medicine. It has outstanding biological properties more than any other cereals which is very useful for the beauty and cosmetic industry (Li et al. 2014) (Fig. 2).

The rice bran with the fats is cold pressed to extract albumin protein which has both nutrients and biological activity used as an ingredient in various cosmetic, beauty, and also in the food products. It has an excellent antioxidant property which makes it useful for the cosmetic products. In addition, it stimulates the collagen creation, nourishes the skin and face, reduces wrinkles, and moisturizes and nourishes the hair to make it smooth and shiny (Zheng et al. 2019; Phongthai et al. 2017) (Fig. 3).

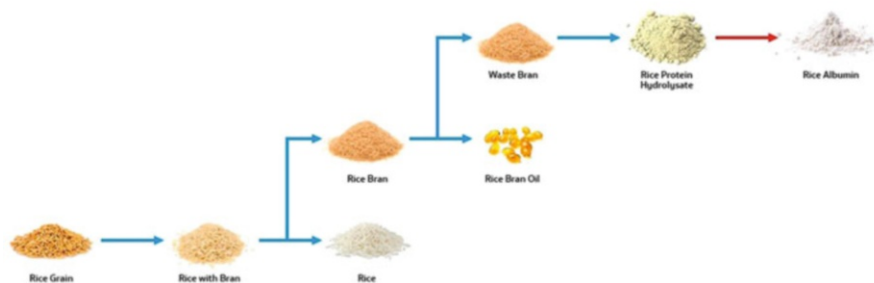


Fig. 2 Extraction of albumin from rice



Fig. 3 Properties of albumin protein: (a) moisturizer, (b) antioxidant, and (c) anti-aging

Albumin and Skin Smoothing

Albumin causes a tightness on the skin with skin smoothing effect because it fills the cracks like putty; the refractive index of the albumin softens the wrinkle lines. The facial skin can be easily stretched by a slight force because of their loose and flabby nature. This nature of the skin is due to the degeneration of the connective tissue, and the network of elastin and collagen fibers is highly altered. The collagen fibers in the dermal structure are degraded heavily whereas the elastic tissue is increased greatly. As the solution gets dried the contraction of the skin occurs which lifts the grooves to the normal level and thereby reduces the wrinkles as that of the surrounding skin (Maidhof et al. 2019).

Dermal Delivery of Albumin in Cosmetic Products

The dermal route of protein administration has various advantages, and they avoid first-pass metabolism, bypass the absorption, avoid the effect of pH and enzymes, and transit time. They can also be made into controlled delivery of proteins and also has an excellent biocompatibility. In this study, they have used inorganic nanoparticles (NPs) such as calcium phosphate nanoparticles (CaP-NPs) and zinc oxide nanoparticles (ZnO-NPs) as enhancers (Kianfar 2021). CaP with a molecular weight (MW) of 310.176 g/mol contains calcium and phosphate and they are essential nutrients as well. ZnO with a MW of 81.408 g/mol acts as an antioxidant, heals the wounds, and also treats other skin disorders (Shokri and Javar 2015). These two inorganic particles have excellent biocompatibility, nontoxic, and cytoprotective, and they are also inexpensive. These inorganic nanoparticles used in this study were not used as drug carriers, rather they are used as enhancing agents. Here, the albumin protein is not attached or loaded into the NPs but they are positioned to the skin layers in which it helps the albumin to pass into the skin. Therefore, the NPs were used as the penetration enhancers and not as the carriers of albumin. Moreover, in this method they have added organic solvents as chemical penetration enhancers (CPEs). They have used six organic solvents as CPEs such as coconut oil (Co), olive oil (Ol), sunflower oil (Su), dimethylsulfoxide (DMSO), liquid paraffin (LP), and tetrahydrofuran (THF). The oils and the rest of organic solvents were used because they can be used safely as they are FDA-approved dermal CPEs. The enhancing mechanism of inorganic NPs and the CPEs were chosen because they have a combination effect to obtain an improved and highest permeation of albumin (Shokri 2014).

Effect of CaP NPs and Solvents Permeation

The albumin is a globular protein with high MW hydrophobic compound which hinders to pass the skin. Lipids present in the subcutaneous (SC) prevents the dermal permeation of molecules. So the enhancing agents such as CPEs and the inorganic

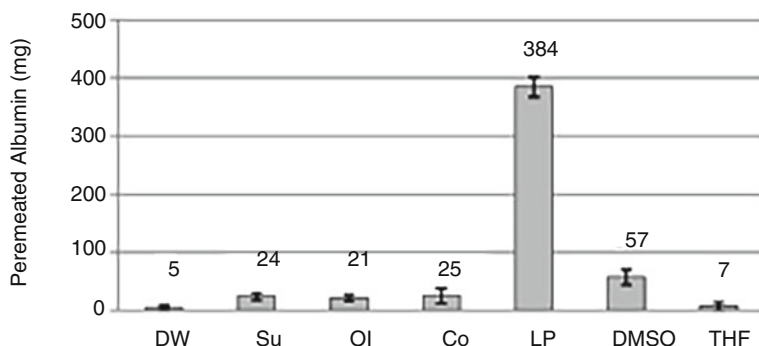


Fig. 4 Permeated amounts of albumin coconut oil (Co), olive oil (OI), sunflower oil (Su), dimethylsulfoxide (DMSO), liquid paraffin (LP), and tetrahydrofuran (THF)

NPs can dissolve and soften the lipid structure and thereby improve the permeation of albumin (Desouza et al. 2013). THF is highly volatile and it has low ability to dissolve albumin and lipids. Su is a mixture of oleic acid (omega-9) and linoleic acid (omega-6) group of oils and 10% of terpenes. OI is the combination of 55–83% of oleic acid and palmitic acid. Co is 91% of saturated fatty acids. These oils slightly increase the permeation of albumin due to their weak capability to dissolve lipids. Also, the albumins can bind to fatty acids which helps in albumin permeation with the presence of solvents. The DMSO increases the albumin permeation more than that of oils because of their ability to dissolve lipids in spite of its volatile nature. When compared to all the solvents used, LP has the increased permeation ability of albumin because it dissolves and softens the lipids much better than the other solvents. Moreover, the LP consists of hydrophobic hydrocarbons which penetrates and deposits in between the hydrophobic chains in the lipid layer of SC and also it improves the albumin permeation by decreasing the consistency of SC lipids (Sahdev et al. 2013). In this study, LP was selected as the best solvent CPEs for the permeation of albumin (Fig. 4). The results of this study indicate that simultaneous use of CaP NPs with each and every one of the solvent leads to increased permeation of albumin than the individual solvents alone. This also exhibits that the increase in the albumin permeation by CaP NPs is proportional to that of each and every solvent. It is specified that the CaP NPs augmenting action was to make stronger the effect of each solvents. Among all the combinations, the combination of CaP NPs and LP increases the permeation of albumin which leads to increase of about 86 mg of albumin (Sun et al. 2010).

Effect of ZnO NPs and Solvents Permeation

Similar to CaP NPs, the ZnO NPs and LP increases the permeation of albumin than other combinations of solvents. ZnO NPs and LP combination leads to increase of about 103 mg of albumin which is considered as the most effective combination.

They have also found that the triple combination such as CaP NPs, ZnO NPs, and LP did not increase the permeation of albumin than the combination of ZnO NPs and LP (Fig. 5). The two combinations were considered to be more effective with the combination of two enhancers ZnO NPs and LP. This enhancing capacity of CaP NPs, ZnO NPs is referred to the nanosize range and also its crystalline state (Shokri et al. 2014). The microscopy images clearly show that it is not only be referred to their penetration into the skin and also it retains on the skin layers for a specified period of time. This particular phenomenon disorders the microstructure of the skin layers and it makes the skin permeable to albumin. The same phenomenon makes the skin permeable to the solvents which in turn improves the permeation of albumin. The triple combination such as CaP NPs, ZnO NPs, and LP leads to a saturation state of permeation of albumin which means it possess the improved enhancing effect but it was not significantly more effective than the combination of ZnO NPs and LP. The combination of the inorganic NPs and LP used in this study can be attributed to the two different mechanisms. Seemingly the two mechanisms could be able to complete the enhancing the effects of permeation of albumin. Figure 6 shows that the

Fig. 5 Permeated amounts of albumin CaP NPs and LP, ZnO NPs and LP and CaP NPs, the ZnO NPs and LP

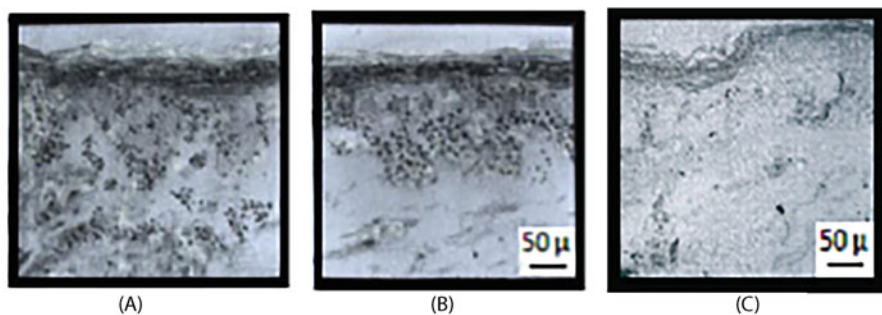
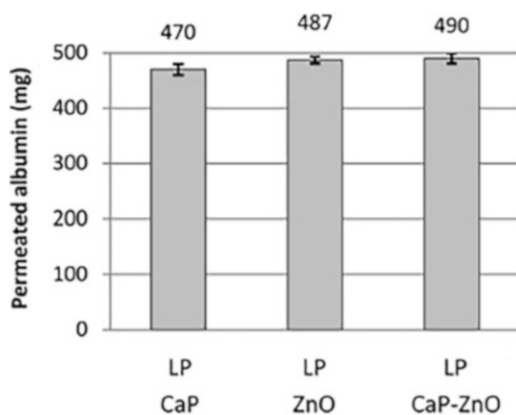


Fig. 6 Histomicrographs of mice skin (a) CaP NPs, (b) ZnO NPs, and (c) untreated skin

skin distribution of CaP NPs spreads in all the layers of the skin whereas the ZnO NPs mostly deposited in the SC and epidermal layer. The concentration of ZnO NPs in the SC layer was more than the CaP NPs and thus the ZnO NPs alter the SC layer than the CaP NPs which in turn enhance the permeation of albumin more than that of CaP NPs. In this study, the results suggest the combination of ZnO NPs and LP to enhance the permeation of albumin effectively in the skin and this dermal delivery of albumin protein is useful in the formulation of cosmetic products (Shokri and Javar 2014).

Human Serum Albumin Cell Carrier Solution for Skin Regenerative Application

Several hours are required for transportation of produced cells to reach the application sites. Here, they have examined four candidate solutions for transporting human keratinocytes. The solutions are normal saline, Saline and human serum albumin (saline with 2.5% HSA), keratinocyte with pituitary bovine extract (PBE-media), and xeno free keratinocyte media. One million keratinocytes were collected from three donors which were suspended in each solution and preserved at 4 °C for 24 h. The cells preserved in the saline and HSA displayed higher viability after 1, 3, and 24 h and the viable cells were seeded in the collagen matrix and cultured it for 48 h. Colonization and adhesion were higher in the cells placed in the PBE media. They have reported saline and HAS as a suitable cell transportation solution to use clinically, and the PBE media is considered to be the alternative one for the transportation of cells (Shahin et al. 2020).

The cellular therapy is the developing and emerging field for the burns and chronic wound management in the clinical therapy. Faster healing and better skin quality are obtained by saline and HAS cultured keratinocytes. The cell therapy field is considered as the Advanced Therapy Medicinal Products (ATMPs) as there a various regulations were updated. The cells have to be manufactured and processed according to the GMP (Good Manufacturing Practice) which is specific to Advanced Therapy Medicinal Products. An example is in the patients who suffer from major burns, the keratinocytes are sprayed on the surgically excised burn wounds and this skin is used for harvesting autologous skin grafts. The medical union has stringent regulations for ATMPs, which include cells cultivation should be fully taken with the GMP compliance (Sarkar et al. 2020). Accordingly, the cells were transported to the clinical site which will administer the cells, and this transportation process takes several hours from the manufacturing place to the clinical site (Burke and Mi 1994). Moreover, the readiness of the patient and team for surgery may need more time and it can also be extended for a period of 24 h. It is always preferred to transport the cells as fresh cells because freezing of cells are associated with decreased number of cells and phenotypes. The keratinocyte cells were kept in the cell carrier solution at 4 °C for 24 h, and the cell count and viability were estimated after 1 h, 3 h, and 24 h. After 24 h, the viable cells and the viability were high in the saline and HSA group compared to other groups. About 2.5% of HSA is added to saline as it is prepared in

the microencapsulated form in poly lysine alginate, and this is considered as a protective cell carrier. In their studies, they have used this HSA as a preservative solution for smooth and endothelial muscle cells and also investigated that 2.5% of HSA is the optimum concentration to preserve the cells at 4 °C for 24 h and 48 h. The HSA overcomes the infectivity problems and is widely used as carrier solution in more complicated drug delivery systems. Albumin is incorporated in the cell culture along with the peptides and growth factors. HSA binds to the outer surface of the cell membrane by adsorption and provides high protection to the cells. The albumin prevents the damage of the cells caused by hydrodynamic stress and is taken by the cells through endocytosis clathrin-coated pits (Lee et al. 2005) (Fig. 7).

The cell culture media was used as the transfer solution for 48 h for the avulsed teeth and after reimplantation the periodontal cells were viable for 12 months. In another experiment they have used four different media to transport keratinocytes for 12 h and found that the morphology was similar before and after transportation. These results confirm that the cells transported at 4 °C for 24 h and 48 h were viable and they can adhere to the cell culture. After 24 h in the transport solution, the cells have equal number of viable cells when resuspended at 37 °C and 5% CO₂ for 48 h. The cells were then stained with the crystal violet solution, and they have the ability to form colonies and also adhere to the cell culture surface. The elution of the stain displayed higher absorbance for the cells in the PBE than the other groups and there was no difference in the CDM and saline + HSA solution. The keratinocytes were characterized by immune staining of the marker CK-14 (Cytokeratin 14) and they were fixed after 48 h. The cells from all the groups look positive for the skin and no difference was observed in the groups. This study recommends the usage of 2.5% HSA in saline solution as the cell carrier for up to 24 h at 4 °C for clinical use. Based on the clinical trial the cell transportation and the cell viability were preserved for use in patients with burn wounds and chronic burn wounds (Xu et al. 2013; Kent and Bomser 2003) (Fig. 8).

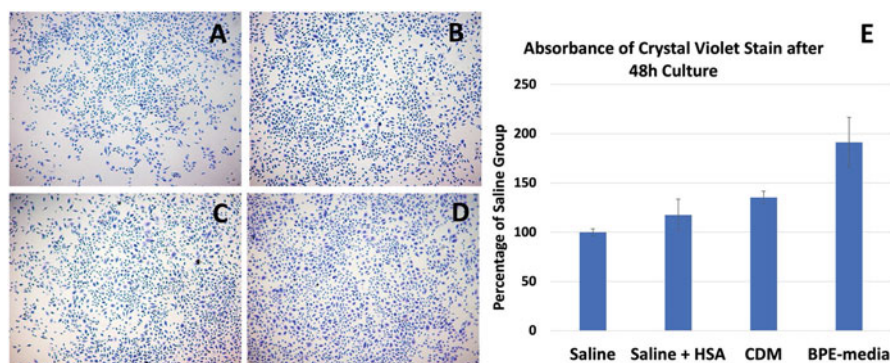


Fig. 7 Crystal violet staining showed adherence and colonization, following 24 h incubation at 4 °C, of keratinocytes kept in saline (a), saline + HSA (b), CDM (c), and BPE-media (d). The elution of the stain (e) was higher in the BPE-media in comparison to the rest of the groups. Also, CDM had higher intensity than saline group. (Reproduced with permission from scientific reports)

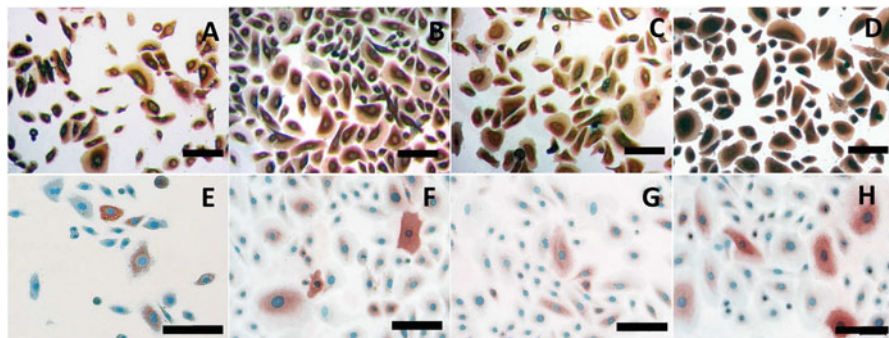


Fig. 8 Immunocytochemical staining for cytokeratin 14 (a–d) and involucrin (e–h). Keratinocytes kept in saline (a, e), saline + HSA (b, f), CDM (c, g), and BPE-media (d, h) showed comparable positive staining for the studied markers. Scale-100 μ m. (Reproduced with permission from scientific reports)

Transdermal Perfusion of Nanoformulation Containing BSA for Cosmetic Applications

It's very challenging to perfuse the large molecules such as proteins in the transdermal route because of the lipophilic nature of the skin. The main barrier function of the skin responsible for the perfusion is stratum corneum which is the outer protective layer and it consists of flat dead cells. They are filled with the corneocytes and lipid bilayers which are impermeable to the large molecular weight compounds. The protein/peptide molecules that are hydrophilic are made into dispersion formulations by making the external layer hydrophobic and thus this dispersions are permeable to the SC layer. Protein nanodispersions with the size range of 210–260 nm of 27 and 40 kDa showed poor permeation into the skin layer. In another study they have used small sphere size of the nanodispersions of about 190 nm which improves the transdermal delivery. This spherical size nanodispersion is useful in improving the permeation of larger proteins for skin vaccination (Martins et al. 2013).

In this study they have used a 66 kDa of bovine serum albumin to attain the size of 162 nm nanodispersion. The albumin (BSA) – fluorescein isothiocyanate (FITC) nanodispersions were manufactured using solid-in-oil technique. They have adopted three different steps in which the sucrose ester surfactant is dissolved in chloroform and BSA-FITC using high pressure homogenization. Then this sample is subjected to lyophilization to remove the water and chloroform to obtain a surfactant–protein complex. After lyophilization, the sample is dispersed in isopropyl myristate by ultrasonication of about 20 kHz to form nanodispersion (Fig. 9). The figure explains the physical appearance of BSA-FITC in the presence and absence of nanodispersion (Martins et al. 2013).

The in-vitro permeation of the prepared BSA nanodispersions in the pig skin was examined using Franz diffusion cells system. The pig skin specimens were collected and the thickness is 66–72 μ m; the follicular structure, collagen fiber arrangement, and content of glycosphingolipids and ceramides of the pig skin resemble that of the

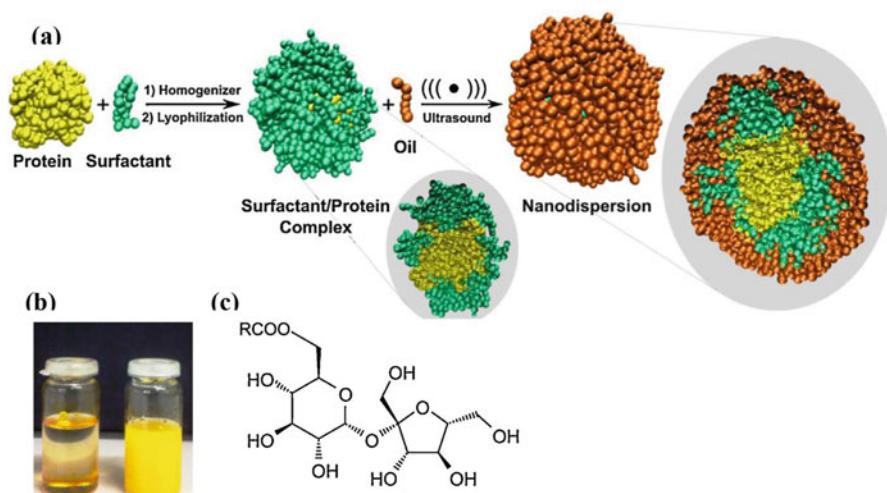


Fig. 9 (a) Schematic preparation method of BSA nanodispersions, (b) appearance of nano-dispersion without sucrose ester, and (c) chemical structure of sucrose ester surfactant

human skin. The specimens of the pig skin were placed between the two compartments in the Franz cells, the donor and the receptor. Then the pig skin was kept for incubation at 37 °C for 48 h and then washed many times to remove the excess particles which had not permeated the skin. The samples were taken for the histological studies, and using confocal laser microscopy the permeation of the skin were studied (Moser et al. 2001). The extent of penetration and BSA nanodispersion localization are illustrated in the 2D and 3D images (Fig. 10). By the image acquisition technique which is the result of acquiring the images multiple times per second for the analysis of diffusion kinetics. From the confocal analysis it is clearly demonstrated that the fluorescence intensity of the BSA nanodispersions is much higher in the SC and the epidermis and as it gets lesser it crosses to the dermis. The permeation results were attained mainly because of the small particles and the hydrophobic coating on the outer layer of the particles. The rate controlling step of permeation to the skin is that it passes through the hydrophobic region of the skin. This study proves that the high molecular weight BSA coated with the surfactant which is hydrophobic can easily penetrate the skin. It gives lot of benefits to the cosmetic industries as it transdermally delivers the large proteins across the skin membrane (Huzil et al. 2011; Schreiner et al. 2000).

Bovine Serum Albumin Loaded Niosomes for Cosmetic Applications

Bovine serum albumin is a biological protein that is widely used in cosmeceuticals and delivery applications, as a suitable carrier because of its non-toxicity, non-antigenicity, and biodegradability. BSA is used as combination with other

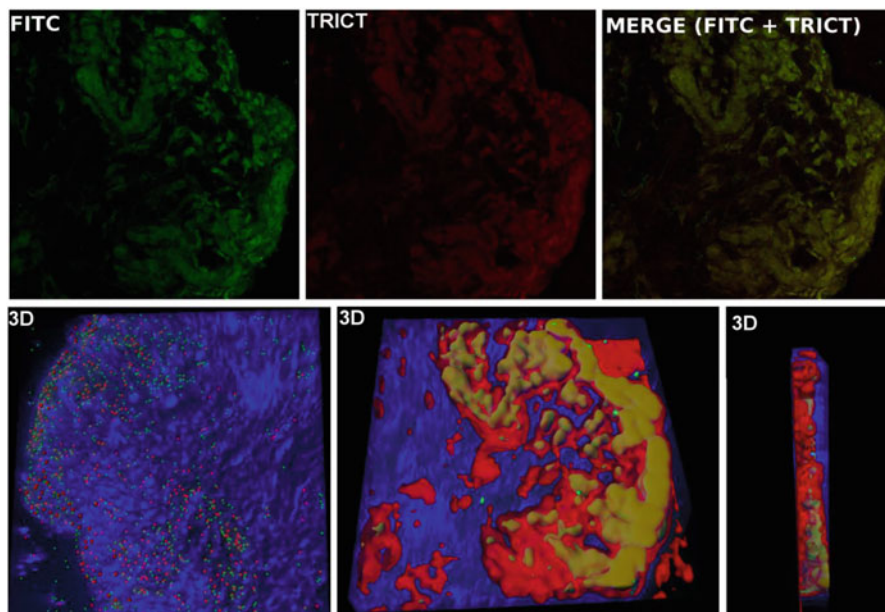


Fig. 10 Confocal images of pig skin crossed by BSA nanodispersion: upper 2D illustration FITC images correspond to the green color with emission maxima at 520 nm emits from FITC-labelled BSA protein and TRICT correspond to the red color with emission maxima at 576 nm emits from TRICT-labelled IPM oil. The Merge images (FITC + TRICT) combine green and red fluorescent signals emitted from the same sample. 3D images represent reconstruction of z-stack confocal images. These images demonstrate a piece of skin marked with blue; the left image represents a particles reconstruction (green particles represent FITC-BSA and red particles TRICT-IPM oil). The central image combine green and red channels (FITC-BSA green and TRICT-IPM oil red channel) and the yellowish color symbolizes the overlapping of both. The right 3D image demonstrates the view of the skin piece from the side where the penetration of FITC-BSA and oil-TRICT is easily detected. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this chapter) (Reproduced with permission from *Colloids and Surfaces B: Biointerfaces*)

ingredients/polymeric systems mainly for increasing the stability and prolonging the effect. The niosomes are vesicles that have high biocompatibility, low toxicity, and is useful for targeted drug delivery and skin tissues. Hydrophilic, hydrophobic, and biomacromolecular agents such as proteins and peptides were encapsulated in the vesicles. The surfactants such as Span and cholesterol are added as additive stabilizing agent, and these are the main components that acts as the nanocarrier for the formulation of niosomes. Encapsulated proteins and growth factors are required greatly in the pharmaceutical and cosmetic industries. The BSA-loaded niosomes were synthesized by the reversed phase evaporation method. The Span 60 with different percentage weights were dissolved in diethyl ether. Simultaneously BSA was dissolved in the deionized water and it is added to the flask by mixing them with the formation of vortex for 60 s. Then it is emulsified using sonication method for

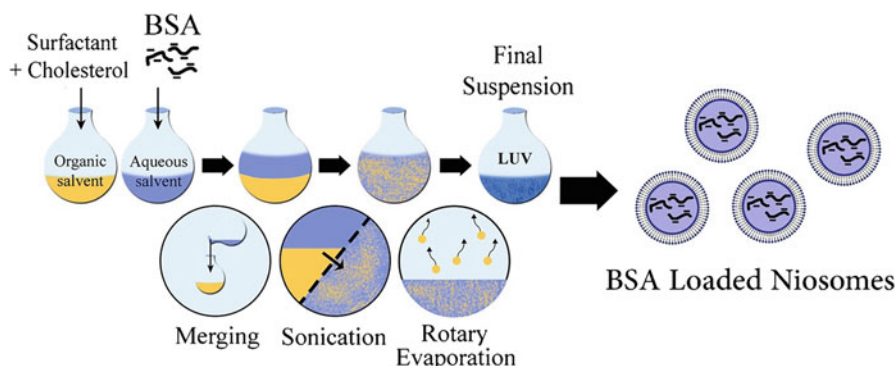


Fig. 11 BSA loaded niosomes. (Reproduce with permission from AAPS PharmSciTech)

3 min in a water bath at 10 °C. The emulsion was kept for evaporation process to remove the excess organic solvents at 40 °C with a rotation speed of about 60 rpm (Fig. 11) (Moghassemi et al. 2017; Mahale et al. 2012).

The in-vitro release profile of BSA-loaded niosomes showed that 30–50% of the loaded BSA was released within the first hour because of the BSA molecules present on the surface. The niosomes with 80% showed a slower release profile because it has created a lower entrapment efficiency which created a difference in the two sides of the niosomes when compared to others. This type of release profile is also obtained for the niosomes containing span 85 and span 80 in which the BSA (30%) released after 3 days. Then the BSA was treated with the MO with a mixture of acidic buffer (pH = 4). BSA along with MO causes a change in color from white to orange, and the colored BSA niosome formulation indicates that the encapsulated BSA niosomes through the optical microscopy studies. They have also studied the absorbance of albumin-MO at 560 nm with different pH values. They have also analyzed using surface plasmon resonance (SPR) analysis to study the binding of MO-albumin and it is stated that one or two MO molecules bind to the albumin molecules at pH 3.6–7.2 (Fig. 12). The sustained release of BSA niosomes were particularly useful for cosmetic, drug delivery, and tissue engineering applications (Nasir et al. 2012; Chaikul et al. 2019).

Chitosan-Ovalbumin Nanogels for Cosmetic Applications

Chitosan and ovalbumin are the two natural macromolecules that are used to formulate nanogels for cosmetic applications. Chitosan and ovalbumin solutions were mixed and pH of the solution was adjusted. Then the solution was stirred and heated till the ovalbumin gets gelled to form a nanogels. In the nanogels formulation, the nanogels core is partly entrapped by the chitosan chains and the remaining chitosan chains form the shell of the nanogels. The prepared chitosan ovalbumin nanogels exhibited excellent stability and didn't dissociate in the pH of 2 to 10.5

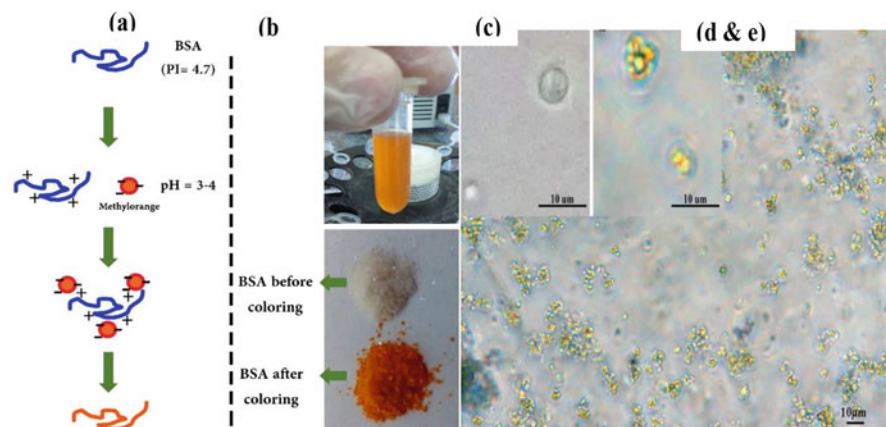


Fig. 12 (a) Scheme showing the mechanism of dyeing of BSA by MO, (b) final colored BSA with MO and light microscopy images of uncolored BSA-loaded niosomes (c) and colored BSA-loaded niosome (d and e)

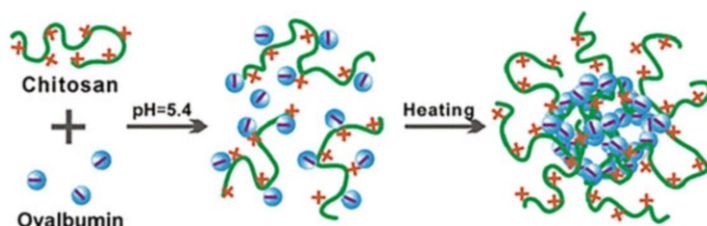


Fig. 13 Chitosan-ovalbumin nanogels for cosmetic applications

(Fig. 13). The size, dispersibility of the preparation, and hydrophilicity and hydrophobicity of the nanogels are pH dependent. The shell structure of the chitosan in the prepared nanogels gives appealing properties. The main advantage of this system is that the nanogels are formed by a convenient and green method of preparation, and there is no separation procedure required. Such exceptional properties make the chitosan-ovalbumin nanogels good candidates for cosmetic industry (Yu et al. 2006; Yin et al. 2012) (Fig. 14).

Protein Formulations for Cosmetic Applications

The protein formulations based on emulsions and dispersions were the most investigated area in the cosmetic industry. The formulations can be used for the delivery of cosmetic agents, transdermal perfusion, and cosmetics application. Various methods have been used to formulate proteins and they are emulsification, self-assembly, coacervation/desolvation, and solid-in-oil dispersions. For the preparation of protein formulations in the micro- and nanoscale, emulsification and dispersibility methods are the commonly

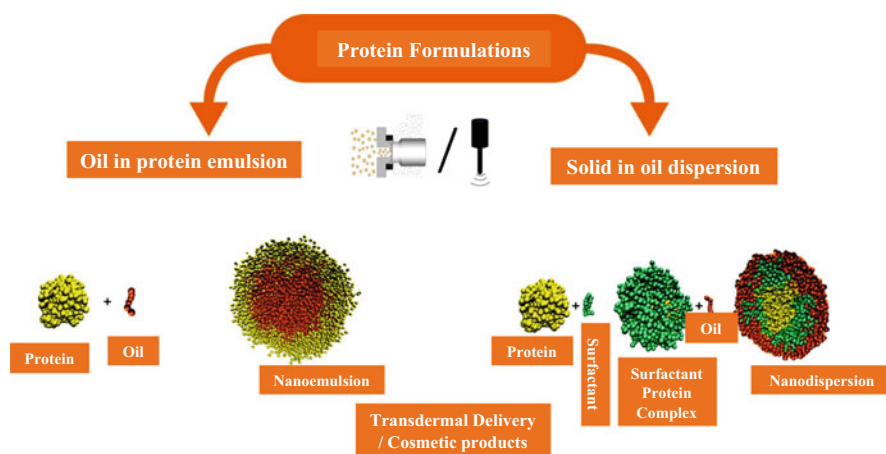


Fig. 14 Protein formulation showing microemulsion and nanoemulsion prepared by mechanical high energy methodology for cosmetic applications. (Reproduced with permission from Trends in Biotechnology)

used approaches. These protein formulations can be used for delivering the constituents through skin permeation (Ye et al. 2013). There are certain factors that impact the behavior of the protein preparations and they are particle size distribution, solubility, temperature, surface tension, ionic strength, and type of emulsifier and concentration of emulsifier. It is a very important step to select the type of emulsifier for the formation of an emulsion and the effective emulsifiers are nonionic, ionic, or mixtures of nonionic surfactants. These emulsifiers lower the interfacial tension and stabilize the emulsion against flocculation and coalescence. The concentration of emulsifiers play a crucial role in obtaining homogeneous emulsions and the protein molecules have spherical and regular surfaces and it has good mobility in the emulsion interface. The penetration into the aqueous phase leads to solvate the hydrophobic portion and it gets adsorbed at the fluid interfaces. The adsorption of proteins on the oil water interface stimulates the changes in the conformation of the structures in the protein solution (Martins et al. 2016). These changes leads to a very structures of albumin in solution. Fig. 15a shows the spheres of BSA prepared at high shear forces by the sonication method, and these high shear forces are responsible for the conformational changes in the protein at the interface of solvent and water. Figure 15b depicts the inner portion of the BSA spheres changes when the RNA is encapsulated with the BSA and it turns more hydrophilic. For the preparation of nanodispersion, sucrose and fatty acid were used as nonionic surfactants in which the hydrophobic part is the outer layer. Because of the high monoester content it forms a hydrophilic sucrose ester and the high esterification leads to lipophilic sucrose ester. The sucrose ester has the low hydrophilic-lipophilic balance (HLB), they have the higher hydrophilic part, and the HLB varies with the rate of esterification of the sucrose. The sucrose ester has a HLB value range from 0 to 20 and this low HLB value designates that they are more hydrophobic in nature (Fig. 15c). They have excellent biocompatibility, biodegradability, and low toxicity and are potentially used in cosmetic formulations (Li Zhai et al. 2013).

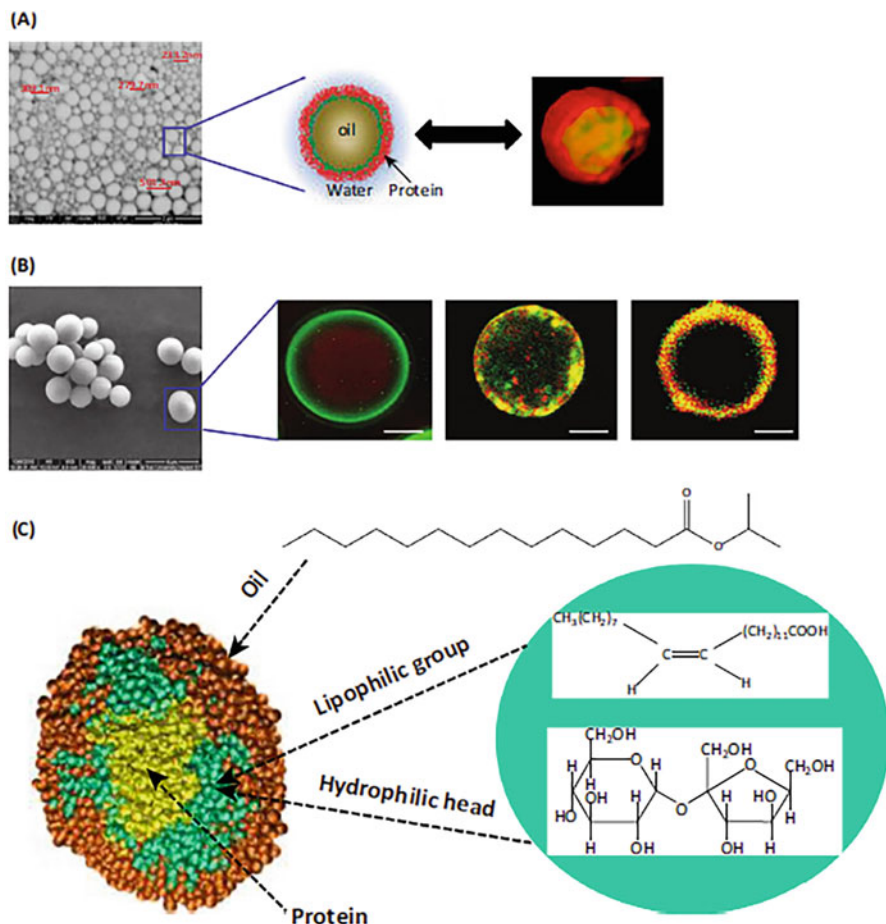


Fig. 15 Protein arrangement at the interface of the microspheres. (a) SEM images of BSA, and it shows that the BSA is at the interface of hydrophilic (red) and hydrophobic phase (green). (b) RNA-loaded BSA microsphere and the confocal microscopy images showing RNA encapsulated in BSA by solid-in-oil dispersion method using sucrose as surfactant. (Reproduced with permission from Trends in Biotechnology)

Serum Albumin for the Treatment of Various Skin Disorders

An invention which has the composition of serum albumin used to treat various skin disorders and it can also provide improved skin protection for the extreme climatic conditions. The amount of albumin used for treating various skin conditions depends on the specific category of the composition. Usually the concentration of albumin used ranges from 1 mg/ml to 90 mg/ml of the gel base. 20 mg/ml of recombinant serum albumin with 10–75% alcohol solution is used as sanitizing solution which is

used for treatment during postoperative incision. This albumin sanitizer is useful in providing antimicrobial protection to the skin. Another albumin preparation used for UV blocking gives sun protection and the ingredients required are serum albumin, para amino benzoic acid, titanium dioxide, zinc oxide, cinoxate, and dicamphor salicylate.

The composition contains recombinant human serum albumin of about 20 mg/ml used as freeze dried form and it is dissolved in the alcohol-gel base which contains propylene glycol, vitamin E, hydroxy ethylcellulose, and sodium hydroxide. It is used as a potential agent for the topical use as per the clinical study. The same composition is used for treating psoriasis which is studied clinically, and this albumin alcohol-based gel was applied in the skin areas. Scaling was followed by the formation of new skin and then after 3 weeks the skin became normal because the psoriasis condition has not reappeared. The same formulation is tested clinically for treating acne and the albumin alcohol gel is supplied with 40 ml tubes. The patients applied the albumin alcohol gel over the acne areas which showed improved skin conditions.

10 mg/ml of recombinant human serum albumin was added to the lotion base and studied clinically by applying this ointment topically on the hands for the treatment of dry skin. Their hands became soft, smooth texture giving a good conditioning and moisturizing of the skin without the presence of oil or greasy nature. The composition contains 10 mg/ml of recombinant human serum albumin, theobroma cocoa, almond oil, cetosteryl alcohol, vitamin E, and sodium hydroxide. 1 mg/ml recombinant human serum albumin was added to the lip balm base which contains bees wax, castor oil, lanolin, and vitamin E. When tested clinically, all of them got a protective effect over the application of lip balm in the chapped lips and found a huge improvement in their lips. This lip balm showed a long-lasting effect by moisturizing and conditioning the lips with soft supple nature (Patent 1988).

Conclusions

In conclusion, albumin has numerous cosmetic applications such as skin regeneration, transdermal delivery, skin lightening, antiaging, anti-wrinkling, antioxidant, moisturizing and conditioning, protection and treatment of dry skin, acne, psoriasis, sagging, elasticity, pigmentation, sun burns, etc. The albumin has numerous skin functions in which it retards the skin aging by various mechanisms, and it directly inhibits the catalytic action of tyrosinase, scavenging of ROS, inhibition of ROS synthesis, improves the cellular antioxidant capacity, maturation of tyrosinase and other melanocytic enzymes, and decrease in the inflammatory responses of the cells. The future studies are needed to improve the clinical efficiency of albumin and its cosmetic formulations, thereby increasing the content in the cosmetic products which in turn can be attained by improving the skin permeation through optimization process. The improved efficiency can be achieved by the combined use of active ingredients albumin derivatives along with different target components for the better treatment of skin pigmentation. During that process, the intracellular melanosome

transfers from the melanocytes to the keratinocytes. Therefore, further studies are very important to identify their analogs and its metabolites action more in clinical aspects.

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Part VII

Future Scope of Biopolymers



Biodegradation of Biopolymers: Reflections Towards Possible Biomagnification 50

Definitions and Assessment

Punniamoorthy Thiviya, Ashoka Gamage, Asanga Manamperi, Sudhagar Mani, and Terrence Madhujith

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P. Thiviya

Postgraduate Institute of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka

e-mail: thiviya904@gmail.com

A. Gamage (✉)

Department of Chemical and Process Engineering, Faculty of Engineering, University of Peradeniya, Peradeniya, Sri Lanka

A. Manamperi

Materials Engineering Department, California Polytechnic State University, San Luis Obispo, CA, USA

Department of Chemical Engineering, College of Engineering, Kettering University, Flint, MI, USA

e-mail: wmanampe@calpoly.edu; amanamperi@kettering.edu

S. Mani

School of Chemical, Materials and Biomedical Engineering, University of Georgia, Athens, GA, USA

e-mail: smani@uga.edu

T. Madhujith

Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka

e-mail: tmadhujith@agri.pdn.ac.lk

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Abstract

Biodegradable biopolymers have gained much attention over synthetic non-biodegradable petroleum-based polymers. Petroleum-based nonbiodegradable polymers are produced in large amounts globally, which end up in the landfill. Their accumulation in the environment can be a major threat to human health and ecology. Shifting to biodegradable polymers can reduce the waste disposal problems associated with conventional petroleum-based polymers. Various biopolymers from natural sources are highly used in many applications, including agriculture, packaging, biomedical, and environmental remediation applications, due to their biodegradability, eco-friendliness, biocompatibility, low cost, and availability. This chapter discusses the type of various biodegradable polymers in terms of classifications, mechanisms, chemical structures, and biodegradability of various biopolymers. This chapter mainly covers the natural biopolymers, including starch, cellulose, pectin, chitin, gellan gum, gelatin, polylactic acid (PLA), and polyhydroxyalkanoates (PHAs).

Keywords

Biodegradability · Biopolymers · Cellulose · Chitin · Gellan gum · Polyesters · Polysaccharides · Starch

Introduction

Petroleum-based nonbiodegradable synthetic plastics, such as polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), and polyvinyl chloride (PVC), are produced globally in large amounts, and only 9% have been recycled. These nondegradable conventional plastics dumped in landfills have accumulated in the environment, and the fragmentation of these plastics into microplastics (less than 5 mm) causes serious ecological and health problems (Agarwal 2020; Ahmed et al. 2018; Maraveas 2020). Microplastics found in the environment can be transported through the food chain and accumulated in plants, animals, and humans. The presence of microplastics in many food products has been reported in literatures, including shellfish, algae, sea salts, fish, drinking water, and few mentions in other foods, milk, beer, fruit, honey, tea bag, chicken, and animal food (Kwon et al. 2020; Pironti et al. 2021). Recycling of plastic waste is also not preferable due to the potential risk of leakages of toxic and synthetic chemicals, such as antioxidants, plasticizers, and stabilizers (Maraveas 2020). Furthermore, the depletion of petroleum and increasing

demand for polymers emphasize the importance of biodegradable polymers from renewable sources (Siracusa and Blanco 2020).

Therefore, there is an increasing demand for natural biodegradable polymers as a substitute for nondegradable petrochemical-based polymers to combat the adverse effect. Biopolymers have many advantages, including complete biodegradability/compostability, recyclable, eco-friendly, noncorrosive in nature, nontoxic, low cost, and abundantly available (Ramesh Kumar et al. 2020; Ray et al. 2021). Biodegradable polymers are generally classified into two groups: natural polymers and synthetic polymers (Godavitame et al. 2017). Natural polymers, including polysaccharides, proteins, etc., are derived from renewable or biological sources (plant, animal, microbial, and marine sources). Synthetic polymers, including polyesters and aliphatic polymers, are chemically derived materials from renewable resources (natural) or nonrenewable (petroleum-based) resources (Balaji et al. 2018; Ramesh Kumar et al. 2020).

Biodegradation of biopolymers undergoes fragmentation or breakdown of polymers into monomers and oligomers through the effect of enzymes and/or other factors (heat, moisture, sunlight) and then complete mineralization of fragmented compounds by the microbial activity, which produces carbon dioxide, water, ammonium, nitrogen, hydrogen, and biomass (Folino et al. 2020).

However, the use of biodegradable plastics has been limited by their higher cost, narrow processing windows, moisture sensitivity, and poor mechanical and barrier properties (Laycock et al. 2017). Generally, polymers are modified or blended with other polymers to enhance the properties of biopolymers, which would change the overall biodegradability.

The present chapter discusses the various types of biodegradable polymers, applications in various fields, mechanisms of the biodegradation process, and biodegradability of various biopolymers. This chapter mainly discusses about polysaccharides (starch, cellulose, pectin, chitin, gellan gum), protein (gluten), microbial polysaccharides (PHAs), and bio-based synthetic polymer (PLA).

Types of Biodegradable Polymers: Classification of Biodegradable Polymers

Biodegradable polymers are generally categorized into two groups: natural polymers and synthetic polymers. Natural polymers can be produced from renewable biological sources, whereas synthetic polymers can be produced from biological resources (renewable resources) or petroleum resources (nonrenewable resources) (Balaji et al. 2018; Doppalapudi et al. 2016).

Biopolymers can also be classified according to the source of raw materials and manufacturing methods (Fig. 1) (Cheng et al. 2021; Ferreira et al. 2016; Ghanbarzadeh and Almasi 2013; Nair et al. 2017).

1. Natural polymers from vegetal or animal biomass – polysaccharides (plant sources: starch, cellulose, hemicellulose, lignin, guar gum, pectin; animal

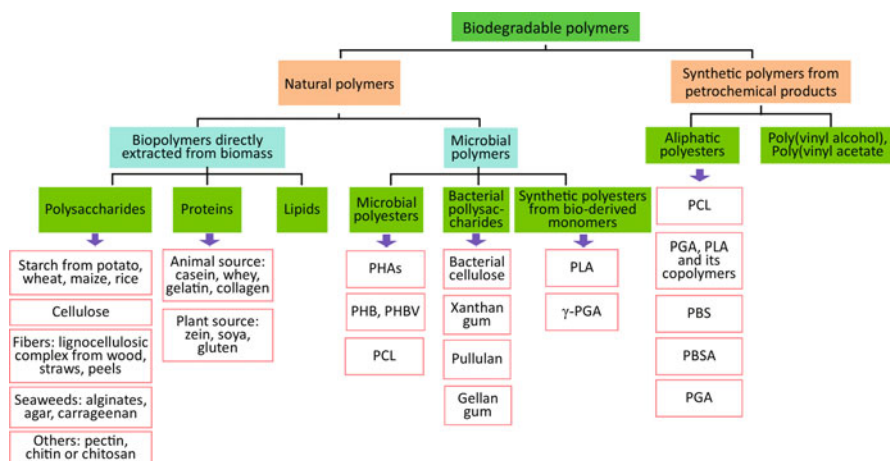


Fig. 1 Classification of biopolymers

sources: chitin and chitosan; marine sources/seaweeds: alginates, carrageenan, agar), proteins (animal sources: collagen, gelatin; plant sources: gluten, soy protein, zein), and lipids (cross-linked triglycerides)

2. Microbial polymers – polyhydroxyalkanoates (PHAs), polyhydroxybutyrates (PHB), poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV), poly(γ -glutamic acid) (γ -PGA), and bacterial cellulose, cellulose, xanthan, gellan, and pullulan
3. Synthetic polymers (chemically synthesized from renewable bio-based monomers or petroleum polymers) – polyesters, such as polycaprolactone (PCL), polyglycolide (PGA), polylactic acid (PLA), and poly(glycolic acid) (PLGA); polyvinyl alcohol (PVA); poly(butylene succinate) (PBS); polybutyl succinic acid-butyl adipate (PBSA); and poly(butylene adipate-co-terephthalate) (PBAT)

Many natural and synthetic biodegradable polymers have gained much attention over the recent past decades. Natural polymers, including polysaccharides (cellulose, starch, pectin, chitosan, gellan gum, alginates, carrageenan, agar), proteins (gluten, gelatin), lipids, and polyesters (PHA, PHB, PHBV, PLA monomers) from various plant, animal, and microbial sources, as well as synthetic polymers, including PLA, PVA, PCL, PBSA, and PBAT, have been widely used to construct many biodegradable materials (Cheng et al. 2021; Ruggero et al. 2020; Udayakumar et al. 2021). Petroleum-based polymers, including PCL and PBS, can also be synthesized by microorganisms (Nair et al. 2017).

Applications of Biodegradable Polymers

Biodegradable polymers have a wide range of potential applications in various fields, including food packaging, agriculture, environmental remediation, and bio-medical (Mohapatra et al. 2017).

Environmental Remediation

Due to the rapid growth in industrialization, various contaminants, such as heavy metals (Ni^{2+} , Cu^{2+} , Co^{2+} , Cd^{2+} , Cr^{6+} , As, Hg, etc.), pesticides, herbicides, fertilizers, oil spills, industrial effluents, sewage, organic pollutants (aliphatic and aromatic hydrocarbons, pharmaceuticals, volatile organic compounds), toxic gases (CO , SO_2 , NO_x), particulate matter, and microbes, have polluted the environment, consequently affecting human health (Briffa et al. 2020; Guerra et al. 2018).

Various biopolymers, including cellulose, lignin, starch, chitosan, alginates, gelatin, guar gum, and their composite films, are used as adsorbents, membrane-filtration, coagulants, and flocculants to remediate the environment (Udayakumar et al. 2021). Nanomaterials such as inorganic (metal and metal oxides, such as Ag, TiO_2 , zerovalent iron NPs [nZVI], etc.), carbon-based (carbon nanotubes [CNTs], graphene), and polymeric-based materials have been used to remove various contaminants from the environment (Guerra et al. 2018).

Chitosan can be used as a flocculent, coagulant, and adsorbent to remove the contaminants, such as heavy metals (Ni, Cr, Zn, Cu, As, Cd, Pb, etc.), dyes, antibiotics, biological contaminants, herbicides, pesticides, and pathogenic microbes, from wastewater and soil (Pal et al. 2021).

A wide variety of biopolymer nanocomposites with a high adsorption capacity, made by incorporation of clay into polymers, including starch, cellulose, alginate, chitosan, gelatin, collagen, and PLA, has been used in the removal of heavy metals, reactive dyes, pesticides, and bisphenol A (BPA) in water and in soil remediation (del Mar Orta et al. 2020).

The incorporation of fillers or reinforcements from natural sources improves the mechanical and thermal properties of starch polymers. Thermoplastic starch incorporated with filler kraft lignin showed improved mechanical and thermal properties of the starch polymer. Furthermore, the presence of kraft lignin polymer was reported to slow down the biodegradation process of thermoplastic starch, which ensures the stability of the polymer. Thermoplastic starch/kraft lignin film can be a suitable material for the removal of methyl orange in wastewater by absorption (de Freitas et al. 2021).

Water streams polluted by dyes affect the availability of safe drinking water. Water contaminated with dye can be a threat to human health due to its long-lasting and resilient properties. Cellulose, chitosan, starch, hemicellulose, and lignin have been reported for their potential to be used as dye adsorbents to remediate industrial dye effluents and contaminated water bodies (Bhatia et al. 2021; Dassanayake et al. 2021). Biopolymers, such as chitosan and alginates, and their blends, in bulk or in nanoparticle (NP) form, can be used to remove heavy metals (As^{5+} , Au^{3+} , Cr^{6+} , Pb^{2+} , Cu^{2+} , Mo^{5+} , etc.) from contaminated water and soil (Almutairi et al. 2021). Chitosan and alginate NPs with *S. linifolium* extract-phycosynthesized Fe NPs exhibited high efficiency as adsorbents for the removal of Cr^{6+} from contaminated water (Almutairi et al. 2021).

Biopolymers, such as chitosan, cellulose, alginate, and many others, incorporated with ZnO nanofillers can be used as bio-adsorbents or photocatalysts for water remediation (Mallakpour et al. 2021).

Relatively high content of different functional groups in lignin, such as aliphatic carboxyl and hydroxyl groups, can act as adsorption sites for dye molecules, including Safranin O, Basic Red 2, methylene blue, Remazol red, Congo red, methyl orange, and many others, through forces, such as electrostatic interactions, dipole-dipole interaction, polar bonds, hydrogen bonds, and π interaction (Bhatia et al. 2021).

Packaging Materials

Biodegradable polymers and their composites incorporated with nanoparticles or other additives (e.g., essential oils, plant extracts) are widely used in food packaging with various functions, including edible packaging film/coating and active (antimicrobial, antioxidant) and intelligent packaging.

Biodegradable bio-nanocomposite film produced from gellan and xanthan gum incorporated with ZnO NPs was shown to improve the mechanical, water vapor and UV barrier, hydrophobicity, and antimicrobial properties against pathogenic bacteria *S. aureus* or *E. coli* (Rukmanikrishnan et al. 2020).

Multilayer antimicrobial film was prepared from gellan gum and chitosan incorporated with thyme essential oil coarse emulsion or nanoemulsion. In comparison with the coarse emulsion, nanoemulsion of thyme essential oil was shown to increase the mechanical flexibility (elongation at break) and UV blocking, as well as the antimicrobial (against *E. coli*) properties of the developed antimicrobial film (Zhang et al. 2021).

The application of natural fibers from hemp and flax fibers as fillers in PHBV was shown to improve mechanical properties. In contrast, wood fibers had no significant effect on PHBV composite (Frącz et al. 2021).

Coffee and rice husk are good sources of cellulosic materials, which were reported in the range of 34–35%. Cellulose fibers and cellulose nanocrystals from coffee and rice are the potential sources of cellulosic reinforcing material which can be used in many applications including food packaging (Collazo-Bigliardi et al. 2018). Brown rice starch-based chitosan film can be a suitable candidate for sustainable food packaging (Hasan et al. 2020).

Films developed using gliadin-rich fractions showed higher transparency and disintegrated when immersed in water and, thus, can be used for edible packaging material. In contrast, the glutenin-rich film had higher tensile strength and water barrier properties, which can be used for packaging of low- and intermediate-water activity foods (Hernández-Muñoz et al. 2003).

Gellan gum-based film was developed with 2-hydroxyethyl cellulose (HEC) and lignin to study the effect of lignin incorporation on the properties of gellan gum film. The incorporation of lignin at 10% considerably improved the tensile strength, elongation at break, contact angle (or surface hydrophobicity), and water vapor permeability properties of the gellan gum/lignin and gellan gum/HEC/lignin-based composite film. Moreover, the incorporation of lignin in gellan gum exhibited high ultraviolet (UV) protection, excellent radical scavenging activities, and non-cytotoxicity (Rukmanikrishnan et al. 2020).

Shen et al. (2022) studied the effect of the incorporation of lipids, such as paraffin wax, beeswax, paraffin oil, and oleic acid, on improving the water resistance of wheat bran cellulose/wheat gluten composite biodegradable film. Incorporation of paraffin wax, beeswax, and paraffin oil at 5–20% was reported to increase water vapor permeability and surface hydrophobicity. In contrast, oleic acid addition was shown to reduce the moisture barrier properties.

Functional ingredients, such as plasticizers and emulsifiers, can be incorporated into the pectin matrix to improve the properties of pectin-based edible films, where plasticizers improve the mechanical properties of pectin and emulsifiers increase stability and adhesion (Espitia et al. 2014).

Chitosan incorporated with apple peel polyphenols at 0.05% exhibited good water barrier, mechanical, antioxidant, and antimicrobial properties, thus having the potential to be used in antimicrobial active food packaging (Riaz et al. 2018).

Adding bio-nanocomposite of Roselle calyx extract (RE) and ZnO NP to chitosan/guar gum film improves the tensile strength and elongation at break and the barrier, antioxidant, antimicrobial, and sensory properties. Therefore, these antimicrobial and antioxidant films by chitosan/guar gum/RE-ZnO bio-nanocomposite can be used to coat Ras cheese for preservation (El-Sayed et al. 2020).

The addition of ZnO nanoparticles in gellan gum/polyacrylamide film improved mechanical, thermal, and barrier (UV and water vapor) properties as well as decreased hydrophilicity. This film also exhibited antimicrobial properties against pathogenic bacteria, including *Bacillus cereus*, *Staphylococcus aureus*, *Cronobacter sakazakii*, *Escherichia coli*, *Salmonella enterica*, and *Salmonella typhimurium* (Balasubramanian et al. 2021).

Paper is a biodegradable, highly recyclable, and compostable material and has been used in many primary and secondary packaging. The porous structure and hydrophilicity of paper material result in low barrier properties for moisture, gases, and aromas, thereby limiting its application in food packaging (Lo Faro et al. 2021). Paper materials are generally coated with PE to improve the barrier properties, which makes them difficult in recyclability and biodegradability. Biopolymers, such as PHBV and PCL, can be used as paper coating material (Lo Faro et al. 2021).

Agriculture

Biodegradable polymers are also highly utilized in agricultural applications, mainly mulching films and plant pots. Mulching films help to increase the crop's yield and quality, improve weed control, and reduce the requirement for water irrigation and pesticides in modern agriculture (Havstad 2020). Various biodegradable biopolymers, including starch, PBAT, PLA, PBS, and their blends, can be used in the production of biodegradable mulching films (Havstad 2020; Serrano-Ruiz et al. 2021).

Plant tissue culture technology, gellan gum, and agar are commonly used gelling agents in culture media. The type and concentration of gelling agents can affect the physical and chemical characteristics of the tissue culture media. The study on the effect of various gelling agents, such as gellan gum and agar, on three different

stages of rice regeneration revealed that medium solidified with gellan gum was most favorable for callus induction and higher root regeneration over agar medium, while shoot regeneration was not affected by the type of different gelling agents (Mohamed et al. 2021).

Biomedical Applications

Many natural and synthetic biopolymers have significant applications in the field of biomedicine, including controlled drug delivery, wound healing, tissue engineering, and organ substitution (Bhatia 2016; Song et al. 2018). Biodegradable polymers derived from natural sources are highly used in biomaterials due to their biocompatibility, low immunological response, low cytotoxicity, nontoxic by-products, and ability to support cell adhesion, proliferation, and regeneration of new tissues. Moreover, degraded products should be nontoxic, easily metabolizable, and excreted from the human body. Various natural polymers, polysaccharides (hyaluronic acid, chitosan), and proteins (collagen, collagen, gelatin, fibrin, elastin, chitosan, hyaluronic acid, and polypeptides) have been utilized to construct scaffolds in tissue engineering (Klimek and Ginalska 2020; Song et al. 2018; Xue et al. 2021). Fibrin and collagen are frequently used as cartilage and other orthopedic repair materials in tissue engineering. The combination of chitosan and alginate in wound dressings. Furthermore, PHA (microbial polyesters), PLA, PGA, and PLGA co-polymers are the most commonly used synthetic polymers in tissue engineering (Bhatia 2016). These synthetic polymers are widely used in various surgical procedures, wound closure (sutures, staples), and orthopedic fixation devices (ligaments, pins, rods, screws, tacks) (Velu et al. 2021). Natural and synthetic polymers, including chitosan, alginates, gelatin, hyaluronic acid, polyester-based polymers (PLA, PGA, and their co-polymer PLGA), and PCL, have also been extensively used in the applications of drug delivery (Jana et al. 2021).

Low-acyl gellan beads were studied for their potential to be used as carriers for oral delivery of meloxicam in the treatment of colorectal cancer. Low-acyl gellan beads showed pH-dependent behavior in swelling and degradation. At low pH, protonation of COO^- groups in low-acyl gellan results in the suppression of electrostatic repulsion between chain and stabilization of gel and vice versa at high pH. Thus, low-acyl gellan beads can be used as carriers for oral delivery of meloxicam, which is intended to release in the distal parts of the gastrointestinal tract by omitting the stomach (Osmalek et al. 2017).

Table 1 shows the applications of various biodegradable polymers.

Mechanisms of Biodegradability

The “biodegradation process” is defined as the decomposition of materials into CO_2 , CH_4 , H_2O , inorganic compounds, or biomass (del Rosario Salazar-Sánchez et al. 2019). Biodegradable plastics can degrade via various mechanisms. Some

Table 1 Different biodegradable polymers in various applications

Polymer	Applications	Characteristics	References
Starch from corn, wheat, rice, and potato/glycerol	Thermoplastic starch film for food packaging	Maximum tensile strength (4.48–8.14 MPa), Young's modulus (116.42–294.98 MPa), elongation at break (35.41–100.34%), water solubility (27.88–32.57%)	Domene-López et al. (2019)
Tapioca starch/glycerol/polyvinyl alcohol (PVA) liquid/glycerol/bamboo fibers	Reinforcement in tapioca starch to improve the mechanical properties	Tapioca starch with chemical treated or untreated bamboo fibers: tensile strength (1.6–4.0 MPa), tensile modulus (61.4–282.6 MPa)	Yusof et al. (2019)
Lignin-based nano-adsorbents	Water treatment	Adsorption of dye, such as Safranin O, Basic Red 2, methylene blue, Remazol red, Congo red, and methyl orange	Bhatia et al. (2021)
Corn starch/cellulose fillers (cellulose fibers or cellulose nanocrystals from coffee and rice)	Potential cellulosic reinforcing material for food packaging	Cellulose nanocrystals from rice (186%) and coffee husks (121%) improved the elastic modulus of thermoplastic corn starch films Tensile behavior such as elastic modulus, tensile strength, and percentage deformation at break of corn starch films, containing or not cellulosic reinforcing agents, was 258–740 MPa, 8.1–13.0 MPa, and 1.9–13.4%, respectively	Collazo-Bigliardi et al. (2018)
Date palm leaf fibers/PVA/corn starch	Food packaging	Addition of fiber (30 wt %) to the composite film increases the tensile, flexural, and impact strength	Ray et al. (2021)
Brown rice starch/chitosan plasticized by palm oil	Food packaging	9.3–15 MPa tensile strength	Hasan et al. (2020)
Gliadin-rich and glutenin-rich fractions of wheat gluten	Food packaging: edible coating and nonedible film	Glutenin-rich films: higher tensile strength, less extensible, low water	Hernández-Muñoz et al. (2003)

(continued)

Table 1 (continued)

Polymer	Applications	Characteristics	References
		vapor permeability – suitable for nonedible packaging films for low- and intermediate-water activity foods Gliadin-rich films: higher transparency, disintegrated in water, lower tensile strength – suitable for edible coatings	
Wheat gluten/xylans	Biodegradable composite film	Xylan can be used as an additive up to 40% (w/w) in wheat gluten films Mechanical and solubility properties are influenced by wheat gluten/xylan ratio, xylan type, drying conditions, and pH	Kayserilioğlu et al. (2003)
Wheat bran cellulose/ wheat gluten film incorporated with lipids	Hydrophobic biodegradable food packaging	Composite film containing 15% bee wax had the lowest water vapor permeability (0.76×10^{-12} g/cm ² ·s·Pa) and acceptable mechanical properties	Shen et al. (2022)
Whole potato flour/ whey protein isolates or bovine gelatin or chicken gelatin	Biodegradable, edible bioplastic materials	Increased the tensile strength (4.43–5.67 MPa), tensile modulus (97.98–127.65 MPa) and elongation (5.63–20.50%)	Omrani-Fard et al. (2020)
Gellan gum (G)/2-hydroxyethyl cellulose (HEC)/lignin (L)	Food packaging applications	Tensile strength (35.5 and 39 MPa), elongation at break (26.1 and 32.5%), contact angle (57.8 ° and 70 °), water vapor permeability (2.40 and 2.18×10^{-9} gm/m ² ·Pas) in G/L- and G/HEC/L-based composite film (L 10 wt %), respectively Also enhance the UV barrier and radical scavenging activities, with a non-cytotoxicity effect	Rukmanikrishnan et al. (2020)

(continued)

Table 1 (continued)

Polymer	Applications	Characteristics	References
Gellan gum-treated sand	Effective removal of Ni^{2+} from contaminated water	Gellan gum (1%) increased the Ni^{2+} adsorption of sand by 150% or greater	Tran et al. (2021)
Gellan/xanthan gum/ZnO NPs	Food, pharmaceutical packaging applications	Incorporation of ZnO NPs increased the tensile strength (22.1–35.5 MPa), thermal stability (82.5–96.2 °C), glass transition temperature (69.9–74.8 °C), contact angle (54.1–60.8 °), and UV barrier and decreased water vapor permeability ($3.83\text{--}2.31 \times 10^{-9}$ g/m ² Pas). Antibacterial activity against <i>S. aureus</i> or <i>E. coli</i>	Rukmanikrishnan et al. (2020)
Chitosan/apple peel polyphenols (APP)	Antimicrobial active food packaging	CS/APP film with 0.25–1.00% APP exhibited good water barrier (14.65–10.01 g·m ⁻¹ s ⁻¹ Pa ⁻¹), antioxidant, and antimicrobial activity against <i>E. coli</i> , <i>B. cereus</i> , <i>S. aureus</i> , and <i>S. typhimurium</i> . In contrast addition of APP decreases the tensile strength	Riaz et al. (2018)
Chitosan/guar gum/Roselle calyx extract and ZnO NP bio-nanocomposites	Antimicrobial film for Ras cheese	Improved mechanical properties and water vapor and oxygen barrier properties. Higher antioxidant and antimicrobial activities against <i>Escherichia coli</i> , <i>Listeria monocytogenes</i> , <i>Aspergillus terries</i> , <i>Aspergillus flavus</i> , <i>Salmonella typhimurium</i> . No significant changes in pH and acidity; enhance the shelf life of Ras cheese coated with developed film	El-Sayed et al. (2020)

(continued)

Table 1 (continued)

Polymer	Applications	Characteristics	References
Gellan gum/polyacrylamide/ ZnO NP	Food packaging	The addition of ZnO nanoparticles increased the glass transition temperature (from 181.8 to 196.3 °C), thermal stability (87.8–96.5 °C), tensile strength (33.5–43.8 MPa), elongation break (24.6–32.8%), contact angle (58–65.6 °), and UV blocking properties (99.2% protection against UVB) and reduced the water vapor permeability ($2.2\text{--}1.6 \times 10^{-9} \text{ gm/m}^2\text{Pas}$) Improved antimicrobial properties against <i>Bacillus cereus</i> , <i>Staphylococcus aureus</i> , <i>Cronobacter sakazakii</i> , <i>Escherichia coli</i> , <i>Salmonella enterica</i> , and <i>Salmonella typhimurium</i>	Balasubramanian et al. (2021)
Low-acyl gellan beads/ meloxicam/Tween®80	Targeted drug delivery	Showed the pH-dependent behavior in swelling and degradation Potential carriers for oral delivery of colon-targeted meloxicam in the treatment of colorectal cancer	Osmalek et al. (2017)
Gellan gum/chitosan/ thyme essential oil coarse emulsion or nano-emulsion (TEON)	Multilayer antimicrobial film for food packaging	In comparison, nano-emulsion of thyme essential oil improved the elongation at break (14.05%) and UV blocking, as well as the antimicrobial properties against <i>E. coli</i> . In contrast, it decreased their water vapor barrier properties and tensile strength	Zhang et al. (2021)
Gellan gum/heat-treated soy protein isolate/ Clitoria ternatea extract	Active and intelligent packaging film seafood freshness	Incorporation of heat-treated soy protein isolates significantly changes hydrophobicity,	Wu et al. (2021)

(continued)

Table 1 (continued)

Polymer	Applications	Characteristics	References
		water vapor permeability, tensile strength, and swelling capacity Antioxidant and antibacterial properties, freshness indicator of seafood based on pH-sensitive color change of anthocyanin	
PHBV/hemp, wood, and flax fibers		Natural hemp fibers in PHBV increased Young's modulus (about 167% increase), tensile strength value (about 21%), and tensile strength (about 62%)	Frącz et al. (2021)
Paper/PCL or PHBV/polyethylene glycol (plasticizer)	Cellulose-based food contact materials/paper coating	In PCL- and PHBV-coated paper: increased water barrier than uncoated and fluorinated paper PCL showed higher grease resistance than uncoated paper	Lo Faro et al. (2021)
Chitosan/alginate NPs Chitosan/alginate/Fe NPs	Removal of Cr^{6+} from aqueous environments	Both were good adsorbents for Cr^{6+}	Almutairi et al. (2021)

biodegradable polymers, including polysaccharides and polyesters (PHAs), can be directly biodegraded by microbes (enzymatic degradation), whereas others are aided and enhanced by natural factors, such as UV, oxygen, and heat (Folino et al. 2020).

Biodegradation of polymers mainly involves chain cleavage, called depolymerization, through enzymatic and nonenzymatic hydrolysis. Depolymerization of polymer produces water-soluble low molecular weight oligomers, dimers, and monomers, which are then assimilated by microorganisms into biomass or mineralized to CO_2 , H_2O , CH_4 , inorganic compounds, and other metabolic products (Ahmed et al. 2018; Laycock et al. 2017). Biodegradation reactions involve both aerobic (produces CO_2 and H_2O) and anaerobic mechanisms (produces CH_4 and CO_2) (Ahmed et al. 2018; del Rosario Salazar-Sánchez et al. 2019).

Moreover, biodegradation occurs by two mechanisms: surface and bulk erosion. Surface erosion, a faster method, occurs by decreasing the thickness of the material when the surface hydrolysis rate is greater than the diffusion rate of water into the polymer. Since enzymes are larger particles than other catalysts like chemicals and free radicals, it is difficult to penetrate the bulk polymers. Thus, enzymatic degradation involves a surface erosion mechanism. Bulk erosion takes place when the water diffusion rate exceeds the rate of the hydrolysis reaction. The typical

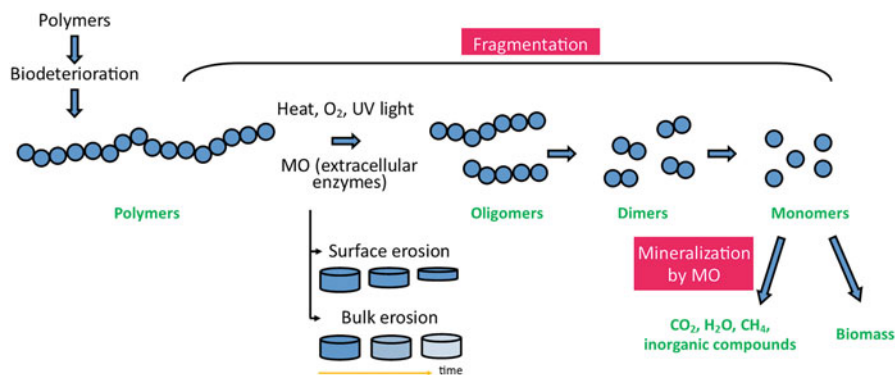


Fig. 2 Schematic diagram for the mechanism of biodegradation

mechanism in polyesters, such as PCL, PGA, and PLA, is bulk degradation. Factors including the chemical nature of the chain, molecular weight, distribution, and crystallinity influence the biodegradation mechanism (Brdlik et al. 2022; Haider et al. 2019; Laycock et al. 2017). Degradation of polymers can occur alone or in combination of surface and bulk erosion mechanisms. Figure 2 illustrates the basic schematic diagram for the mechanism of biodegradation.

Some of the hydrolytic enzymes that are involved in polymer hydrolysis are amylolytic enzymes such as α -amylase and lysozyme (hydrolysis of starch and chitin, respectively), lipase (hydrolysis of ester bonds and aliphatic and aromatic polyesters [PHAs, PCL, PLA, and a PET]), PHA depolymerase (hydrolysis of PHAs), pronase, proteinase K, bromelain (hydrolysis of α -ester bonds such as in PLA), and β -glucosidases (conversion of cellobiose into glucose) (Laycock et al. 2017).

Various blends and composites of biopolymers have been used over the last two decades to counteract the weaknesses of pure polymers, such as rapid degradation rate, poor mechanical and barrier properties, and moisture sensitivity (Jiang et al. 2020; Rosseto et al. 2019). However, the biodegradation rate varies based on the composition of the blend and the compatibilizer added (Dammak et al. 2020). Furthermore, the biodegradability of a material depends on the polymer characteristics (molecular weight, size and shape, crystallinity, surface properties, type of functional groups, additives, biosurfactants) and the environmental or exposure conditions, such as type of microorganisms, enzyme characteristics, nature of pre-treatment, UV radiation, oxygen content, humidity, moisture content, temperature, and pH under which the degradation occurs (Ahmed et al. 2018; Folino et al. 2020; Ruggero et al. 2020).

Biodegradability can be evaluated by various methods depending on the conditions of the test system, measuring weight reduction, CO_2/CH_4 /biogas production, O_2 consumption (biological oxygen demand [BOD] or the ratio of BOD/ThOD [theoretical oxygen demand]), disintegration (loss of visibility in the final compost), loss of mechanical properties, surface erosion, observing surface morphological

changes using microscopic techniques, and reduction of molecular weight using spectroscopy (Folino et al. 2020; Ruggero et al. 2020).

Biodegradation of Biopolymers

Polysaccharides

Various polysaccharides from different sources, such as plants (guar gum and gum acacia), animals (chitosan), microorganisms (xanthan gum), and seaweeds (alginate), are used to prepare hydrogels for various food and nonfood applications (Das and Giri 2020). Cellulose and starch are the primary polysaccharides in the applications of various materials. Other than these, more complex polysaccharide-based polymers, including xanthan, curdlan, pullulan, and hyaluronic acid, produced by bacteria and fungi, also have drawn increasing attention nowadays (Ghanbarzadeh and Almasi 2013).

Starch-Based Biopolymers

Cellulose and starch are the main polysaccharides highly used in many applications. Starch is a hydrocolloid biopolymer, and it is the cheapest and abundantly available polysaccharide (Vroman and Tighzert 2009). Starch is a heterogeneous polysaccharide-based polymer that is composed of two types of molecules: amylose and amylopectin. Amylose is a linear, crystalline polysaccharide made up of α -1,4-D-glucopyranose units, whereas amylopectin is a branched and amorphous polymer made up of short chains of α -1,4-D-glucose units linked by α -1,6 glycosidic bonds (Fig. 3) (Jiang et al. 2020; Olatunji 2016; Vroman and Tighzert 2009).

Starch is a storage carbohydrate synthesized in both lower and higher plants and stored in leaves, roots, tubers, and seeds. Typically, starch is accumulated as water-

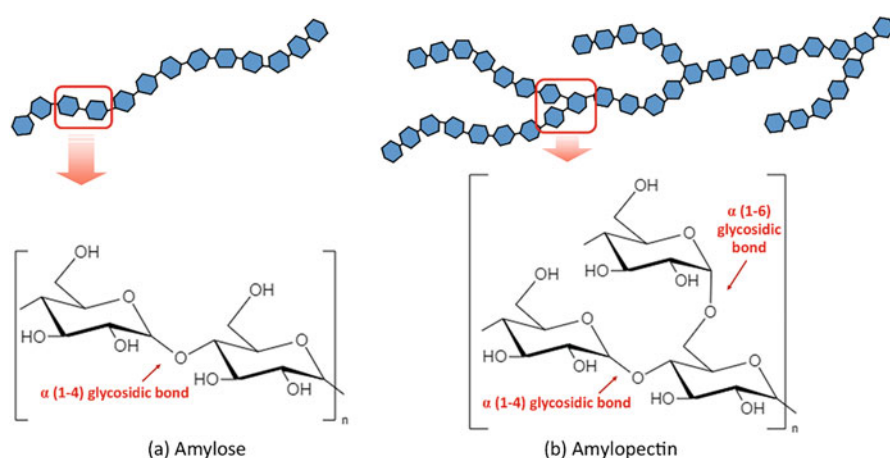


Fig. 3 Chemical structure of starch molecules: (a) amylose and (b) amylopectin

insoluble particles known as starch granules. The internal structure of the starch granules is conserved between organs and species. The molecular weight of amylopectin is higher (10^6 – 10^8 gmol^{-1}) than the amylose molecules (10^5 – 10^6 gmol^{-1}). Amylopectin is the most abundant polysaccharide, accounting for 70–80%, which varies with plant species and organs (Balaji et al. 2018; Compart et al. 2021; Ghanbarzadeh and Almasi 2013).

Though starch is generally insoluble in water and alcohol, it can be irreversibly dissolved in the presence of heat through a process called gelatinization (Olatunji 2016). Thermoplastic starch (TPS) is a gelatinized starch-based material used in many applications (Jiang et al. 2020). The gelatinization process involves granular swelling, destruction of crystalline structure, partial separation of amylose and amylopectin, and molecular solubilization at high temperatures (Jiang et al. 2020). Gelatinized starch can recrystallize or retrograde at low temperatures (Vroman and Tighzert 2009).

Starch can be extracted from various plant sources, mainly from maize, corn, wheat, potato, cassava, barely, and rice (Balaji et al. 2018; Domene-López et al. 2019). Starch has been used in adhesives, paper, clothing, cosmetic creams, food thickener, packaging, and environmental remediation (Mallakpour et al. 2021; Olatunji 2016).

As shown in Fig. 4, biodegradation of starch takes place through hydrolysis at the α -1,4 and α -1,6 acetal link by enzymes and yields nontoxic products. Amylases hydrolyze the α -1,4 link, while glucosidases hydrolyze the α -1,6 link. Chemical modification of starch, such as acetylation and plasticization (substitution of hydroxyl groups) or blending with other molecules, may influence biodegradability (Nevoralová et al. 2019; Vroman and Tighzert 2009). Amylopectin degrades faster than amylose due to the branched structure (Olatunji 2016).

Cellulose-Based Biopolymers

Cellulose, $(\text{C}_6\text{H}_{10}\text{O}_5)_n$, is the main component of the plant cell wall, which has rigid nature and is the most abundant polymer globally (Olatunji 2016). Cellulose is a linear homopolymer of β -D-glucopyranose linked by β -(1,4) glycosidic linkages (Fig. 5). The number of repeating units in a cellulose chain varies with the origins: about 10,000 units in wood while 15,000 in cotton (Rosseto et al. 2019).

Modified cellulose by physical and chemical methods is used in many applications, such as fillers, coating films, laminates, papers, packaging, textiles, optical films, sorption media, viscosity regulators, and building materials, as well as advanced functional materials (Heinze 2016; Motaung and Liganiso 2018).

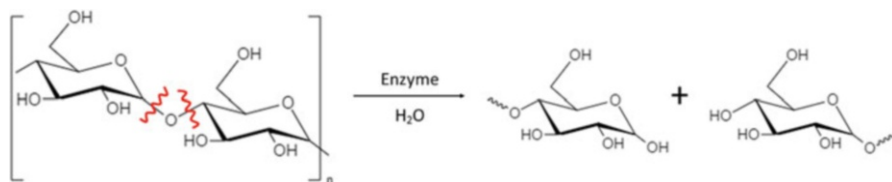
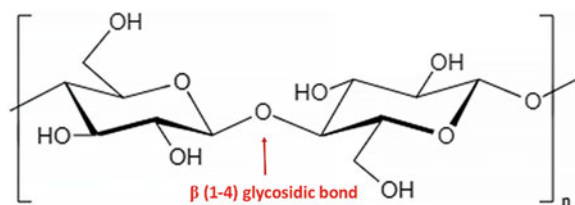


Fig. 4 Hydrolysis of starch

Fig. 5 Chemical structure of cellulose



Cellulose chains stack parallelly via intra- or intermolecular hydrogen bonding and aggregate into microfibrils (5–50 nm in diameter and several microns in length). Arrangements of cellulose chains form two regions in microfibrils: (1) highly ordered (crystalline) and (2) less ordered (amorphous) regions. These microfibrils are further aggregated to form cellulose fibers (Moon et al. 2011; Shahabi-Ghahfarrokhi et al. 2020).

Cellulose is a highly crystalline structure and high molecular weight polymer and possesses distinct properties, including high tensile strength and insolubility in most solvents. Due to the infusibility and insolubility, cellulose is generally modified into various derivatives to improve its processability. The most widely used cellulose derivatives are ethers, methylcellulose (MC), hydroxypropyl methyl cellulose (HPMC), and ester carboxymethyl cellulose (CMC) (Shahabi-Ghahfarrokhi et al. 2020; Vroman and Tighzert 2009).

Wood, cotton, straw, coffee husks, rice husks, bamboo pulp, stalks, pineapple leaf fiber, coconut husk, mango seeds, banana peels, flax, hemp, and many other materials are good sources of cellulose (Collazo-Bigliardi et al. 2018; Olatunji 2016). Plant or vegetal cellulose is generally accompanied by hemicellulose, lignin, and other molecules. Apart from these plant sources, cellulose can be produced by seaweed, fungi, and some species of bacteria, genera *Gluconacetobacter*, *Agrobacterium*, *Pseudomonas*, *Rhizobium*, and *Sarcina*, and notably by the species of *Komagataeibacter* spp. (formerly *Acetobacter* and *Gluconacetobacter*). Bacterial cellulose is an extracellular polymer, although it is chemically equivalent to plant cellulose, found in pure form, that is free of by-products such as lignin, pectin, hemicellulose, and other lignocellulosic compounds. Bacterial cellulose has a high degree of polymerization and a highly crystalline structure. The high purity of bacterial cellulose enables its direct utilization with minimal processing in biomedical and other applications, such as drug delivery systems, wound dressings, surgical materials, scaffolds, and tissue/organ regeneration (Heinze 2016; Swingler et al. 2021). Both vegetal and bacterial cellulose is widely exploited for various applications, including food, paper, packaging, biomedical, and environmental remediation (Collazo-Bigliardi et al. 2018; Olatunji 2016; Swingler et al. 2021).

Pectin

Pectin is a gel-forming polysaccharide found in the plant cell wall at a higher level (up to 30%). Pectin is a heteropolysaccharide, mainly, at least 65%, comprising galacturonic acids linked at the O-1 and O-4 positions. Three major pectic polysaccharides have been identified: homogalacturonan, rhamnogalacturonan I, and

rhamnogalacturonan II. About 65% of pectin is homogalacturonan, having a linear homopolymer of α -1,4-linked galacturonic acids, in which some carboxyl groups are esterified with methanol at C-6 and/or acetyl groups at C-2 and/or C-3 hydroxyl group (Fig. 6). About 20–35% of pectin is rhamnogalacturonan I, which contains repeating units of α -D-1,4-galacturonic acids and α -L-1,2-rhamnose in the backbone. The side chains of arabinans, galactans, or arabinogalactans are attached at C-4 of the rhamnose residues. Rhamnogalacturonan II is the most structurally complex pectic polysaccharide that makes up about 10% of pectin, which consists backbone of galacturonic acids with complex side chains attached at C-2 and C-3. These side chains may consist of arabinose, galactose, apiose, fucose, rhamnose, acetic acid, glucuronic acid, galacturonic acid, and xylose. It is assumed that all these three pectic polysaccharides are covalently cross-linked as they are difficult to separate without using chain-cleavage agents such as enzymes and chemical treatments (Wusigale et al. 2020; Zdunek et al. 2021) (Fig. 7).

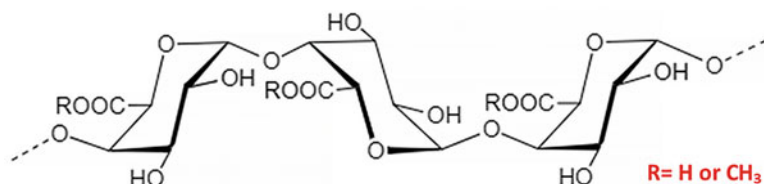


Fig. 6 Chemical structure of pectin

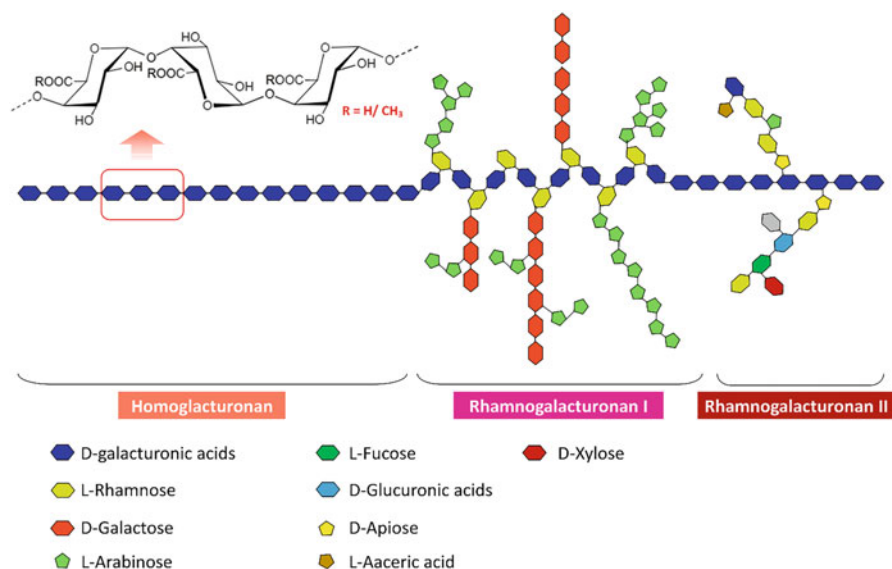


Fig. 7 Diagram of pectin chain containing three main pectic polysaccharides, homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II

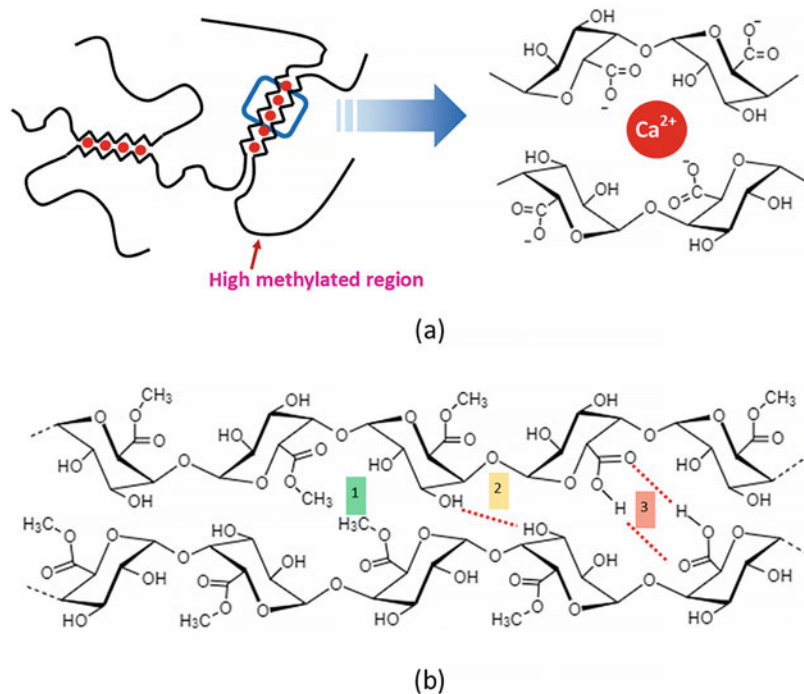


Fig. 8 Mechanism of pectin gelation: (a) LMP (egg-box model) and (b) HMP. 1, hydrophobic interactions; 2, the hydrogen bond between hydroxy groups; 3, the hydrogen bond between carboxylic acid groups

Galacturonic acids and rhamnose are the backbones of each major pectic polysaccharide. DE, acetyl esterification, and molecular weight of pectin vary with the source and extraction methods. Some galacturonic acids are partially esterified with methanol at the C-6 carboxyl group and occasionally with acetyl groups at C-2 or C-3 hydroxyls (Fig. 8). Degree of esterification (DE) is the percentage of carboxyl group esterified with methanol, i.e., methyl-esterified galacturonic acid units. Pectin can be categorized into two types based on the DE: high methoxyl pectin (HMP) (DE > 50%, that is, more than half of the carboxyl groups are esterified) and low methoxyl pectin (LMP) (DE < 50%) (Martău et al. 2019; Rubio-Senent et al. 2015; Shahabi-Ghahfarrokhi et al. 2020).

DE has significantly influenced the solubility, gelling, and film-forming properties of pectin. HMP gelation occurs in conditions of reduced electrostatic repulsions and water activity. HMP forms a thermally reversible gel in the presence of co-solutes such as sucrose or glucose at acidic pH. In HMP, the cross-linking of two pectin chains involves a combination of hydrogen bonds and hydrophobic interactions between the methyl esters. Sugars reduce water activity due to their hydrophilicity, thus promoting hydrophobic interactions between pectin macromolecules. On the other hand, LMP gel formation is independent of pH and sugar conditions. LMP forms a thermally

irreversible gel in the presence of cations (Ca^{2+}), which act as a bridge between two carboxyl groups of different adjacent pectin chains via electrostatic interactions. This mechanism is known as the egg box model, similar to the alginates gel (Basu et al. 2017; Espitia et al. 2014; Ishwarya and Nisha 2022).

Pectin is the most extensively studied polysaccharide, mainly obtained from citrus peel, apple pomace, and sugar beet (Zdunek et al. 2021). Furthermore, pectin can be extracted from food industry by-products, such as fruit and vegetable pomaces, coffee pulp, olive oil by-products, and many others (Martău et al. 2019; Reichembach and de Oliveira Petkowicz 2020; Rubio-Senent et al. 2015).

Pectin hydrogels are widely used in the applications of food ingredients/additives, food packaging, biomedical, controlled bioactive delivery, health management, and many other applications (Ishwarya and Nisha 2022).

Chitin

Chitin is a structural polysaccharide, naturally found in the exoskeleton of insects and mollusks, the shell of crustaceans (shrimp, crab, and lobster), and the cell wall of fungi, mainly in the classes of Basidiomycetes, Ascomycetes, Zygomycetes, and Deuteromycetes. Chitin is also a highly hydrophobic linear polysaccharide of animal origin (Abo Elsoud and El Kady 2019; Olatunji 2016).

After cellulose, chitin is the second most abundant biopolymer in nature. The chemical structure of chitin and chitosan is similar to that of cellulose. The hydroxyl group at C-2 of cellulose monomer, β -(1-4) linked D-glucose, has been replaced with the acetamide group in chitin and chitosan. Chitin is a linear nitrogenous polysaccharide consisting of β -(1,4)-linked-N-acetyl-D-glucosamine (N-GlcNAc) monomers. Chitosan is the derivative of chitin. Chitosan is a linear biopolymer of β -(1-4)-linked-D-glucosamine (GlcN) units. Chitosan is the modified form of chitin, which is obtained from partial alkaline N-deacetylation (Figs. 9 and 10) (Abo Elsoud and El Kady 2019; Gonil and Sajomsang 2012; Martău et al. 2019).

Chitosan is a natural, water-soluble derivative of chitin. Chitosan, a deacetylated form of chitin, can be obtained from chitin by deacetylation through alkaline hydrolysis. Chitin and chitosan can be differentiated by the degree of acetylation (or degree of N-deacetylation). In general, a polymer having a degree of acetylation above 50%, containing more than 50% of N-acetyl-D-glucosamine units, is called as chitin. The polymer with less than 50% N-acetyl-D-glucosamine units, a degree of acetylation less than 50%, is referred to as chitosan. Commercially available chitosan is composed of homopolymers containing various fractions of N-acetyl-D-glucosamine and D-glucosamine residues and has a degree of N-deacetylation between 70 and 100% (Gonil and Sajomsang 2012; Rosseto et al. 2019). Moreover, hydrolysis of chitin under severe conditions, such as of HCl or chitosanase enzymes, breaks the β -(1-4) glycosidic linkages (depolymerization) and produces low molecular weight oligosaccharides. Repeated hydrolysis with HCl (Francis et al. 2021; Martău et al. 2019). Repeated acid hydrolysis transforms the chitin into chitin nanoparticles (Elsabee et al. 2020).

Commercially, chitin is obtained from shellfish sources such as crabs and shrimp (Gonil and Sajomsang 2012). Marine wastes, such as by-products of seafood processing skin, head, tails, shells, scales, and spine, are potential materials for

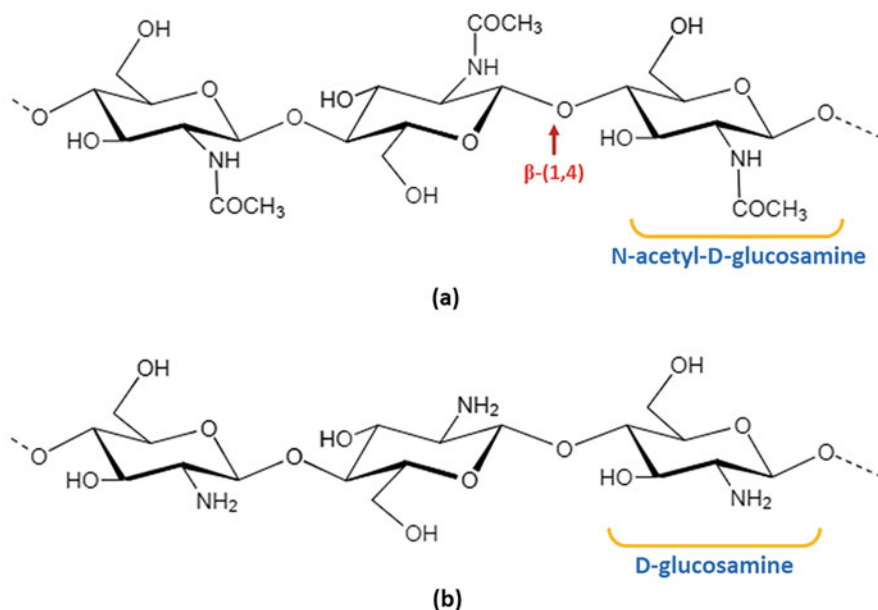


Fig. 9 Chemical structure of (a) chitin and (b) chitosan

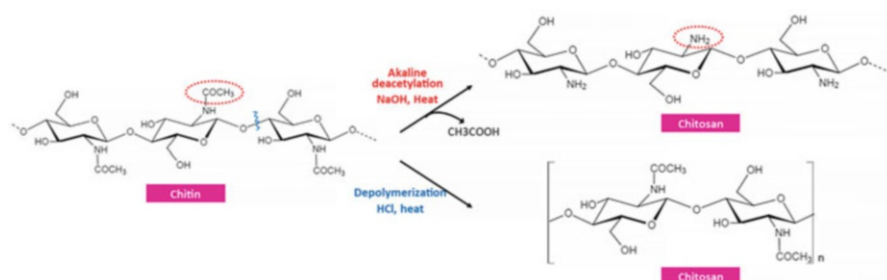


Fig. 10 Chemical preparation of chitosan from chitin

extracting chitin and chitosan. Chitin, chitosan, and their derivatives are used in a wide range of applications, including food, food packaging, biomedicine, pharmaceuticals, cosmetics, agriculture, personal care products, textiles, and the environmental sector (Elsabee et al. 2020; Rosseto et al. 2019).

Gellan Gum

Gellan gum is a linear, anionic water-soluble exopolysaccharide secreted by the bacterium *Sphingomonas elodea* (previously known as *Pseudomonas elodea* or *Aeromonas elodea*). Gellan gum is a high molecular weight (around 5×10^5 Da) compound, which is composed of linear tetrasaccharide repeating units of β -1,3-D-glucose, β -1,4-D-glucuronic acid, and α -1,4-L-rhamnose in the molar ratio of 2:1:1

with two acyl groups, acetate and glycerate, which are bound in the same glucose adjacent to glucuronic acid (Ferreira et al. 2016; Prajapati et al. 2013; Rukmanikrishnan et al. 2020). Furthermore, gellan gum is available in two forms: (1) high-acyl gellan gum, native form, and (2) low-acyl gellan gum. Low-acyl gellan gum can be obtained by deacetylation of native gellan gum through alkaline hydrolysis at high temperatures (Fig. 11) (Das and Giri 2020; Ferreira et al. 2016).

The gel-forming mechanism of both high-acyl and low-acyl gellan gum is associated with disorder-order transition, that is, gellan gum forms a disordered coil structure at high temperature and ordered double helix structure on cooling. Further aggregation of helices forms a three-dimensional network. Aggregation and gelation of helices are inhibited by electrostatic repulsion between the helices due to the presence of negatively charged glucuronate carboxyl groups. Reduction of electrostatic repulsion can be achieved by reduction of pH and/or salt (Morris et al. 2012).

High-acyl gellan gum forms weak gels when cooling a hot solution at low pH. Aggregation of helices is determined by the presence of acyl group or degree of deacetylation. Low-acyl gellan gum forms gel in the presence of cations, such as Ca^{2+} , Mg^{2+} , Na^+ , and K^+ , and promotes the aggregation of double helices of gellan molecules to form a three-dimensional network. Compared to monovalent cations, divalent cation ions greatly promote the aggregation and gelation process. High-acyl gellan gum produces soft, elastic, and thermo-reversible gels ($<100^\circ\text{C}$), while low-acyl gellan gum produces harder, nonelastic brittle, and thermostable gels

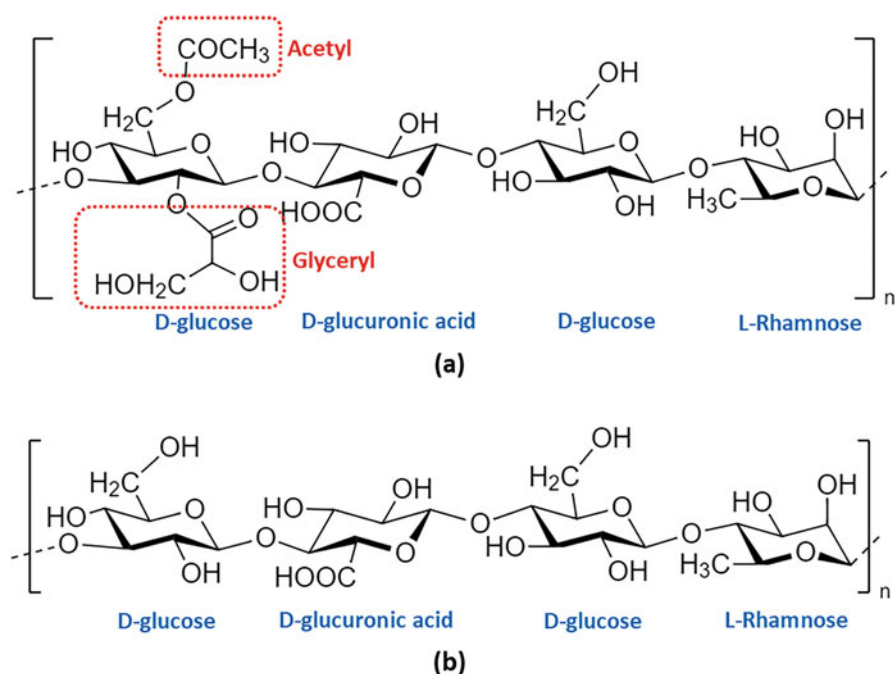


Fig. 11 Chemical structure of gellan gum

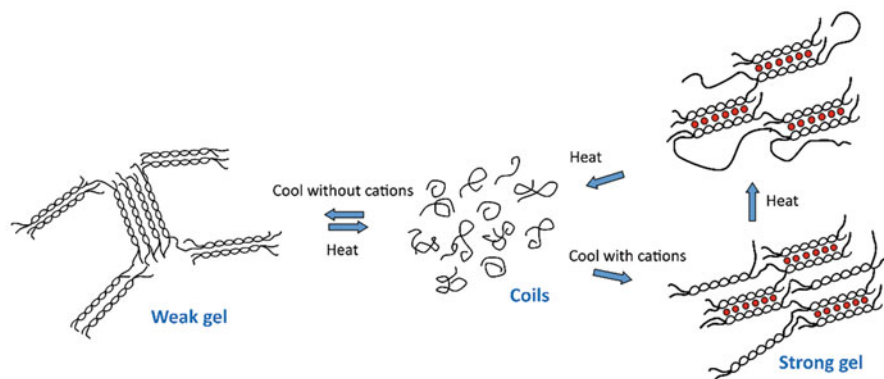


Fig. 12 Schematic diagram for the gelation of gellan gum

(Bacelar et al. 2016; Ferreira et al. 2016; Sworn and Stoubly 2021). The pH, sugars, acyl substituent, ion type, and ion concentration influence the gel formation and its properties (Kang et al. 2015). Figure 12 shows the schematic diagram for the gelation mechanism of gellan gum.

Gellan gum has wide applications in many fields, including food, cosmetics, pharmaceutical, biomedical, tissue engineering, and environmental bioremediation, due to its biocompatibility, nontoxicity, stability, and biodegradability. They are used as a stabilizer, gelling agent, thickening agent, binders, carriers for controlled drug release, coagulants, lubricants, and film formers for packaging and hydrogel-based filters (Das and Giri 2020; Prajapati et al. 2013; Rukmanikrishnan et al. 2020; Zia et al. 2018).

Proteins: Glutens

Gluten has drawn much interest in nonfood applications due to its viscoelastic properties and low water solubility (Lagrain et al. 2010). Wheat protein consists of functional (albumins, globulins) and storage protein, which is also known as prolamins. Gluten is the primary storage protein of wheat grains. Wheat gluten proteins consist of monomeric gliadins (28–55 kDa) and polymeric glutenins (500–1000 kDa). Glutenin represents 40–50% of wheat endosperm protein, and 40–45% is gliadin. Gliadin proteins are a complex mixture of polypeptides and are classified as α/β -, γ -, and ω -gliadins on the basis of mobility at low pH in gel electrophoresis. Glutenin has two subunits: high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits. Gliadins, such as α/β - and γ - gliadins, have casein in their amino acid sequence and form intrachain disulfide bonds. In contrast, ω -gliadins lack in cysteine residues. Glutenins consist of aggregated proteins linked by the formation of intrachain and interchain disulfide bonds (Dybka-Śtepień et al. 2021; Kuktaite and Ravel 2020; Mahan et al. 2018; Wieser 2007).

Considering the secondary structure, α/β - and γ -gliadins mainly consist of α -helices and β -sheets, while ω -gliadins mainly contain β -turns (Kłosok et al. 2021).

The secondary structure of LMW glutenin is similar to α -/ β - and γ -gliadins. However, not like gliadins, LMW glutenin can form intermolecular disulfide bonds. HMW glutenins have three distinct domains: non-repetitive N-terminal, repetitive central, and C-terminal domains. The N- and C-terminals contain α -helices, and the repetitive central domain consists of overlapping reverse β -turns that form a loose spiral structure. HMW glutenins form extended rodlike confirmation (Johansson et al. 2013).

Glutenins and gliadins are interacted with each other to form a gluten network via covalent (intra- and interchain disulfide bonds) and noncovalent bonds (ionic bonds, hydrogen bonds, hydrophobic bonds, and van der Waals forces) (Abedi and Pourmohammadi 2021; Kłosok et al. 2021). Structural variation of gluten constituents (glutenin and gliadins), amino acid composition, and their interactions determine the unique characteristics of gluten. Gluten consists of high levels of glutamine and proline amino acids and low levels of charged amino acid residues (Abedi and Pourmohammadi 2021; Lagrain et al. 2010). Since nonpolar glutamines are found in gluten proteins in higher amounts, they represent a high noncovalent hydrogen-bonding potential between glutenin and gliadin (Lagrain et al. 2010). Covalent disulfide bonds between gluten proteins play an important role in further polymerization and rheological properties. Despite the fact that cysteine residues are found in lower amounts in gluten (about 2% of the total amino acid composition), they play a major role in gluten network formation by promoting cross-linking by disulfide bonds (SS bonds) and disulfide/sulfhydryl (SH/SS) interchange reactions which increase both tensile strength and elasticity of gluten (Abedi and Pourmohammadi 2021; Lagrain et al. 2010). A small increase in the number of disulfide bonds can have a significant structural effect and enlarge the gluten network (Lagrain et al. 2010).

The processing of film coatings or other protein-based materials mainly involves three main steps: (1) breaking noncovalent and covalent intermolecular bonds using physical or chemical modifications, (2) arranging and orienting mobile polymer chains, and (3) formation of new intermolecular bonds and interactions stabilizing the three-dimensional network (Lagrain et al. 2010).

The process of gluten network development involves hydration, depolymerization, and re-polymerization. Hydration of gluten along with mechanical forces is essential to unfold and modify the initial conformation of gliadin and glutenin chains (increasing β -turns and hydrated extended structures). Hydrated gliadins contribute to the viscosity and extensibility of the dough, and hydrated glutenins contribute to dough strength and elasticity. Generally, gluten is considered a “two-component glue,” in which gliadins act as a “plasticizer” or “solvent” for glutenins (Kłosok et al. 2021; Wieser 2007).

Mixing/shearing and temperature play a crucial role in forming a three-dimensional protein network stabilized by the interchain disulfide bonds, hydrophobic, or isopeptide bonds. Shearing or mixing causes protein denaturation, changes in secondary structure, and increases the molecular mobility of gluten protein, which enhances the exposure of reactive sites and consequently increases the cross-linking reactivity of gluten proteins. Mechanical stress also causes depolymerization by cleavage of disulfide covalent bonds resulting in increased free sulfhydryl group (SH) groups. Heating below the gelation temperature (up to 70 °C) facilitates the formation of the gluten gel network through hydrogen and ionic bond disruption and enhances

hydrophobic interactions. Heating at critical temperatures leads to higher disulfide (SS) bond formation in glutenin by oxidation of the sulfhydryl group (SH). Further increase in temperature leads to glutenin-gliadin interaction by SS bond formation through SH/SS interchange reactions (Abedi and Pourmohammadi 2021; Lagrain et al. 2010). Fig. 13 depicts the gel schematic diagram of gluten network formation.

Wang et al. (2017) investigated the chemical interactions and protein conformation in the heat-induced wheat gluten gelation mechanism. The study also showed that wheat gluten unfolds during heating and aggregates at temperatures of 80–90 °C. The ionic bond and hydrogen bond, and free SH content, were shown to decrease when the temperature was increased from 25 to 90 °C. A decrease in SH

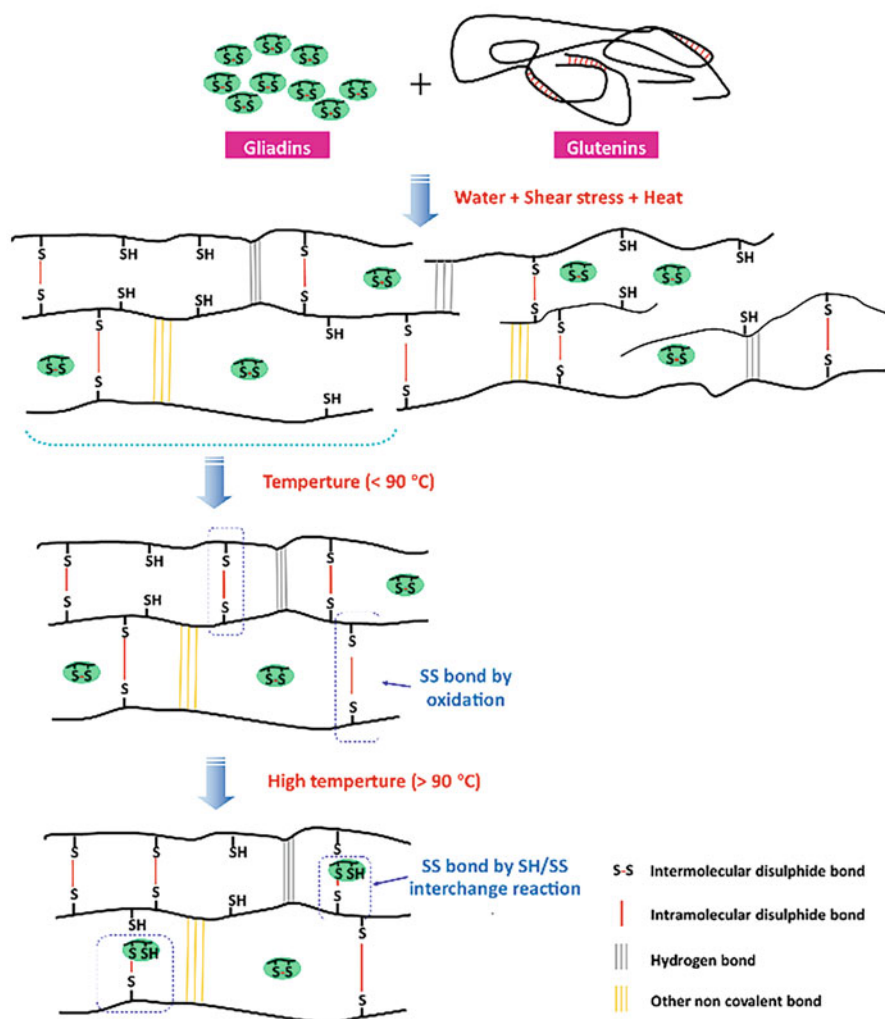


Fig. 13 Schematic diagram of gluten network formation

content can be attributed to inter- and intramolecular disulfide bond formation by oxidation upon unfolding of gluten protein and exposure of SH groups. Random coil content increased at 50 °C, whereas intermolecular β -sheets increased at 90 °C due to gluten aggregation. This study suggested that wheat gluten is unfolded and reorganized during heating, resulting in exposure of hydrophobic and SH groups. Upon further heating gluten network is formed by disulfide and hydrophobic bonds, while ionic and hydrogen bonds have little effect (Wang et al. 2017).

Both nucleophilic and radical reactions were hypothesized to play an important role in gluten aggregation (Auvergne et al. 2008). Mechanical cleavage of the SS bond in gluten leads to the formation of thiol radicals (Fig. 14d) (Abdel Aziz et al.

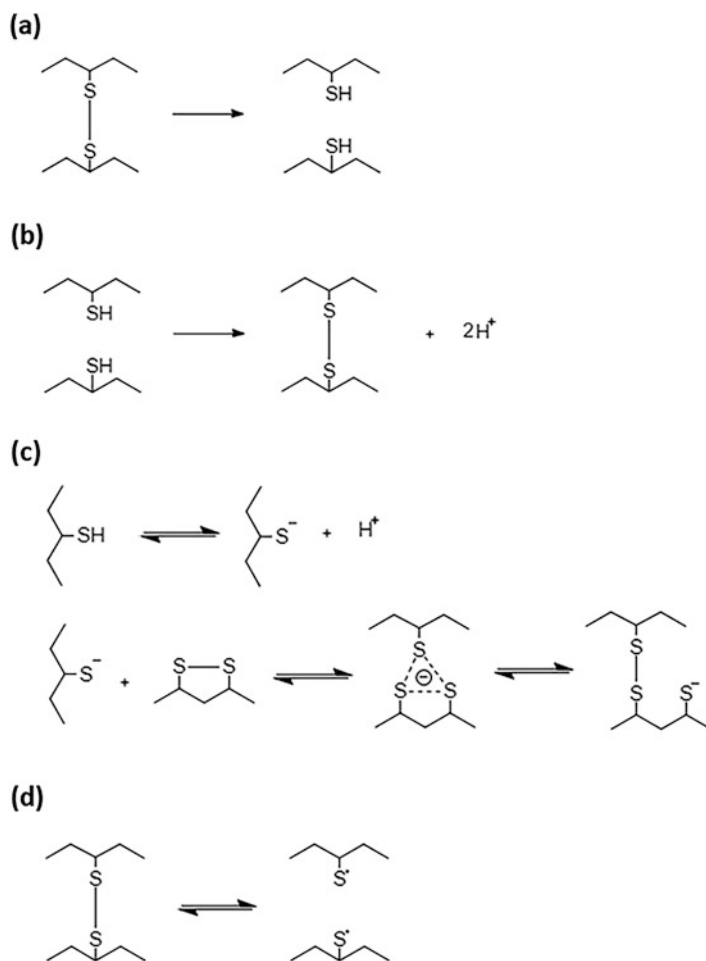


Fig. 14 Reaction between gluten chains: (a) mechanical cleavage, (b) oxidation and disulfide bond formation, (c) SS/SH exchange reaction, and (d) free radical formation

2018). The influence of mixing on thiol and disulfide groups in the wheat gluten network was studied. During mixing, a transient increase in free SH (thiols) groups was recorded, but the formation of total free SH groups dropped with increasing temperature. The free SH group formation may result from the reduction or scission of disulfide bonds. Moreover, when mixing continues at a particular temperature, the result indicated an irreversible loss in thiol equivalent groups, i.e., a net decrease in SS groups higher than free SH increase. This may be attributed to the formation of sulfoxyl compounds by oxidation of thiyl radicals (S^\bullet) that might form during mechanical scission of disulfide bonds (Morel et al. 2002).

Gluten is a plant protein that can be obtained from the by-product of starch fabrication. Among other proteins, gluten has a unique feature, viscoelastic properties. Due to the thermoplastic properties and its potential for chemical modification, gluten can be used to develop a range of materials for food and nonfood applications, such as composite materials, foams, edible film, food packaging material, and films for agricultural uses (Abedi and Pourmohammadi 2021; Domenek et al. 2004; Dybka-Śtepien et al. 2021; Kuktaite and Ravel 2020). Protein films, including wheat gluten films, have good oxygen barrier properties at low relative humidity (Hernández-Muñoz et al. 2003; Pirsá and Aghbolagh Sharifi 2020). Protein films can be used to pack many foods, especially beans, fruit kernels, and cashews (Pirsá and Aghbolagh Sharifi 2020).

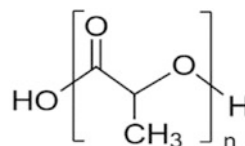
Since gluten is a thermoplastic material, gluten polymer can be processed through the extrusion technique, a well-established technique used in commercial synthetic plastic films (polyethylene). This makes them suitable for industrial application (Jiménez-Rosado et al. 2019).

Synthetic Polymers from Bio-derived Monomers: Polylactic Acid (PLA)

Polylactic acid (PLA) is a thermoplastic, biodegradable synthetic polymer widely used in many applications. PLA is a linear, aliphatic polyester polymer obtained through the polymerization of the D-/L-lactic acid monomer produced by bacterial fermentation of polysaccharides or through chemical synthesis (Grujić et al. 2017). The ratios of L- and D-isomers influence the properties of PLA (Garrison et al. 2016) (Fig. 15).

Lactic acid-based biodegradable polyesters have gained much attention, mainly in medical applications, as they are degradable in the human body and then eliminated by kidney filtration and bioassimilation, as well as in the environment by

Fig. 15 Chemical structure of PLA



microbes (Elsabee et al. 2020, p. 1). PLA has also been used in agriculture (sustained delivery of pesticides and fertilizers, seed protection), drug delivery, tissue engineering, and packaging (Vert 2015).

The lactic acid precursor is obtained from renewable resources and fossil oil (Vert 2015). Lactic acid can be produced from renewable sources, starch, and sugars, using lactic acid fermentation bacteria, such as *Lactobacillus*, *Streptococcus*, *Pediococcus*, and *Sporolactobacillus*. PLA polymers are synthesized by direct polycondensation of lactic acid and ring-opening polymerization (ROP) of lactide, esters of lactic acid (Dubey et al. 2017; Masutani and Kimura 2014; Vert 2015). PLA is commercially produced by ring opening polymerization of lactide (Garrison et al. 2016).

By hydrolysis of ester bonds, PLA is broken down into smaller water-soluble fragments (monomers or oligomers) that can facilitate uptake into microorganisms. Subsequent microbial activity further breaks down the smaller fragments into CO_2 , H_2O , CH_4 , and other metabolic end products, while a portion of the carbon is converted into biomass (Garrison et al. 2016). Microorganisms mainly bacteria, such as *Amycolatopsis*, *Lentzea*, *Kibdelosporangium*, *Streptoalloteichus*, and *Saccharothrix*, and fungi *Tritirachium album* were reported in PLA biodegradation (Garrison et al. 2016).

Two common degradation pathways of PLA are (1) hydrolytic chain scission and (2) β -C-H hydrogen transfer of PLA (main chain scission). As shown in Fig. 16, the hydrolytic degradation of PLA produces hydroxyl- or carboxyl-terminated PLA end groups, while β -C-H hydrogen transfer of PLA produces carboxylic acid end groups and vinyl esters (Scaffaro et al. 2019).

Microbial Polymers

Various polysaccharide-based biopolymers, including xanthan from (*Xanthomonas campestris*), gellan (*Sphingomonas paucimobilis*), bacterial alginates (*Pseudomonas*

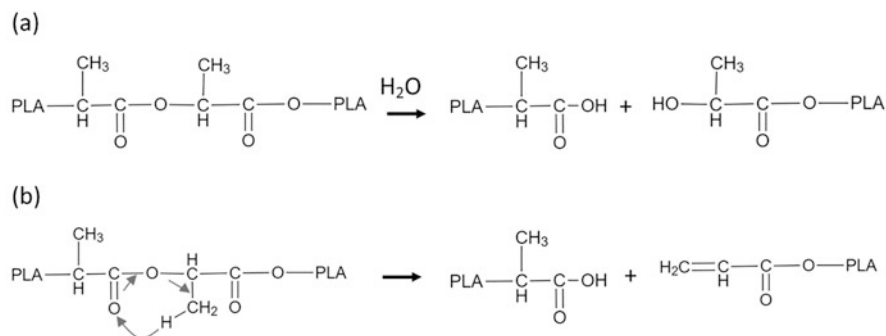


Fig. 16 Two common degradation pathways of PLA: (1) hydrolytic chain scission and (2) β -C-H hydrogen transfer of PLA

spp. and *Azotobacter vinelandii*), bacterial cellulose (*Acetobacter xylinum*), and hyaluronic acid (*Streptococcus equi*), are produced from various microbes (Banik et al. 2000). Other than these, microbes produce polyesters such as PHAs (PHB, PHBV) and lactic acid monomers for PLA (Ferreira et al. 2016).

Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates (PHAs) are microbial-based aliphatic polyesters synthesized as water-insoluble intracellular granules by various microbes, mainly by bacteria, such as *Bacillus* species, in response to environmental stress. PHAs, such as poly-3-hydroxybutyrate (P3HB), poly-4-hydroxybutyrate (P4HB), polyhydroxyvalerate (PHV), and poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV), are produced by various microorganisms, such as *Bacillus megaterium*, *Pseudomonas aeruginosa*, *Pseudomonas putida* (previously known as *Pseudomonas oleovorans*), *P. stutzeri*, *Staphylococcus* spp., and *Cupriavidus necator*. PHAs have various types and are classified into three groups based on the length of the carbon chain: short-chain (3–5 carbon monomers), medium chain (6–14 carbon monomers), and long-chain PHAs (>14 carbon monomers) (McAdam et al. 2020; Mohapatra et al. 2017; Muneer et al. 2020; W. Meereboer et al. 2020). Long-chain PHAs are rare in nature. Fig. 17 shows the general structure of the repeating unit of PHA, where R denotes the functional group, n varies from 1 to 4, and × represents the number of repeating units in the polymer chain (Muneer et al. 2020). Monomer length and molecular weights of PHAs are determined by bacterial strain and growth medium conditions (Garrison et al. 2016).

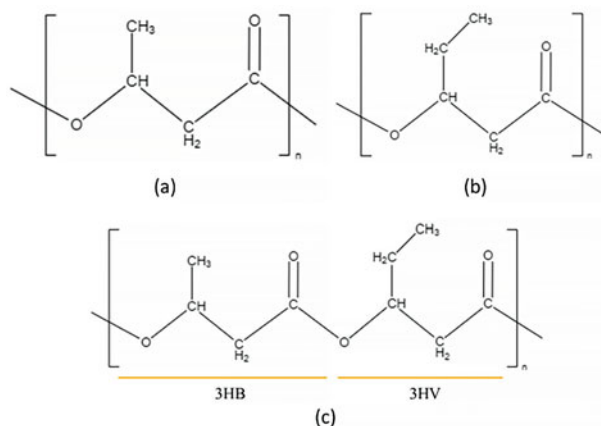
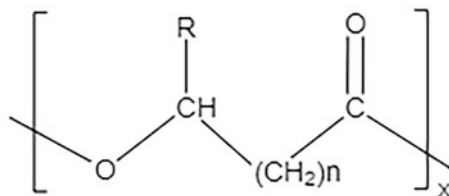


Figure: General chemical structure of the repeating unit of PHAs. (a) PHB, (b) PHV, and (c) PHBV

PHB is the most common homopolymer of PHAs. PHB has a highly crystalline structure due to its linear structure. PHB has higher permeability barrier properties than synthetic polymers (PE and PP), is more rigid and less flexible than PP, and has higher biodegradability. Therefore, PHB has potential applications in food packaging (McAdam et al. 2020). PHBV is the co-polymer of PHV and PHB polymer. PHBV has better mechanical properties than PHB, including lower brittleness and stiffness and higher tensile strength and elongation at break (Frącz et al. 2021).

Fig. 17 General chemical structure of the repeating unit of PHA



PHAs such as PHB and its co-polymer PHBV are highly used in commercial applications due to their biocompatibility, nontoxicity, and negligible cytotoxicity and show biodegradability in all aerobic and anaerobic environments (Mohapatra et al. 2017; Meereboer et al. 2020). PHAs are water-insoluble thermoplastic polymers. PHA-based flexible food packaging is widely used for oily foods, such as marinated olives, cheese, and nuts, frozen foods, and organic foods (Grujić et al. 2017). Other applications include agriculture mulching films, 3D printing materials, tissue engineering, medical implants, drug delivery carriers, textile, nonwoven materials, and animal nutritional supplements, as well as new types of biofuels from PHA polymers (Mohapatra et al. 2017).

PHAs, microbial-derived polymers, biodegrade into CO_2 and water. PHAs are biodegradable by soil bacteria, such as *Bacillus*, *Xanthomonas*, *Stenotrophomonas*, *Pseudomonas*, *Acinetobacter*, *Variovorax*, *Schlegelella*, and *Azospirillum*, and molds, such as *Acremonium*, *Penicillium*, *Verticillium*, *Paecilomyces*, and *Zygosporium* (Mohapatra et al. 2017). These degrading microorganisms produce extracellular PHA depolymerase, which converts the polyesters into water-soluble oligomers and monomers that are used as a carbon source by the organisms (Anjum et al. 2019).

Composites and the Biodegradability

Biodegradable plastics generally have limitations, such as hydrophilicity, brittleness, poor mechanical and barrier properties, and higher degradation rate in their pure form; thus, chemical and physical modifications and various blends and composites are widely used to combat these limitations (Garrison et al. 2016; Jiang et al. 2020; Rosseto et al. 2019; Tarique et al. 2021; Meereboer et al. 2020). The effect of these treatments on biodegradability should be studied.

Biodegradable plastics can potentially lose their biodegradability when blending into polymer composites. Therefore, biodegradation testing is required to ensure that no chemical interactions hinder the overall biodegradation rate (Meereboer et al. 2020). Furthermore, degradation of biodegradable polymers takes place only under certain environmental conditions (temperature, humidity, light, oxygen availability, and microorganisms). Therefore, the impact of disposal of these biodegradable polymers on the environment should be studied (Bagheri et al. 2017). The

biodegradation of many natural polymers and composite polymers has been mentioned in many literatures.

A major fraction of whole potato flour is starch, about 80%, and the rest is protein. However, whole potato flour-based bioplastics have brittle behavior and poor mechanical properties. The study showed that incorporating proteins with potato starch greatly improves the mechanical properties. This may be due to the proteins in combination with starch forming a stable three-dimensional network through strong hydrogen bonds and intermolecular interactions (Omrani-Fard et al. 2020).

Natural polymers are not thermoplastic, with only a few exceptions (gluten). Structural polysaccharides, including cellulose, have strong inter- and intra-interactions, which prevent thermal processing in unmodified cellulose. However, thermoplasticity of natural biopolymers, such as cellulose, lignin, and chitin, can be induced by various chemical and physical modification techniques, including plasticization or polymer blending (Müller et al. 2019).

Chemical modification through acetylation of thermoplastic starch can impact biodegradability. A study revealed that thermoplastic acetylated starch materials showed lower biodegradability than thermoplastic non-acetylated starch (2 mm pieces, i.e., 100 mg) when buried at 58 °C for 90 days. Further, biodegradation of acetylated thermoplastic starch decreased with the degree of acetylation. The hydrophilicity of acetylated starch is decreased with the degree of acetylation process due to the substitution of hydroxyl groups, which in turn lowers the biodegradability (Nevoralová et al. 2019). Plasticization process also significantly affects the biodegradability of polymers (Nevoralová et al. 2019).

The combination of thermoplastic cassava starch and the polylactic acid composite film was shown to have 65% of biodegradability in 32 days under aerobic composting conditions at 58 °C \pm 2 °C. Based on the analysis of changes in the chemical structure, TPS/PLA blended polymers undergo three phases of biodegradation: fragmentation; then disintegration, mainly by the effect of abiotic hydrolysis; and mineralization by the compost microbiota (del Rosario Salazar-Sánchez et al. 2019).

Starch/polyhydroxyurethane hybrid film was recorded with the highest biodegradability with weight loss of about 88% when buried in soil for 120 days. Moreover, the presence of cellulose nanocrystals reduces the potential of biodegradability (about 44% after 60 days) of the nanohybrid film (starch/polyhydroxyurethanes/cellulose nanocrystals) (Ghasemlou et al. 2022).

Ray et al. synthesized biodegradable composite film by date palm leaf fibers reinforced with PVA/corn starch matrix and tested the biodegradability by burying the materials in farmland soil at 10 cm deep under ambient conditions (30 °C and 79–80% RH) for 6 weeks. Composite material containing 40 wt% date palm leaf fiber showed 32% of weight loss, which is lower than the value recorded for PVA/corn starch (95%). Further, it was observed that increasing the fiber content reduces the biodegradability of the composite film, which may be attributed to the presence of relatively low-biodegradable cellulose in higher levels in fiber (Ray et al. 2021). de Freitas et al. observed that the incorporation of kraft lignin as fillers in thermoplastic starch film reduces biodegradability (de Freitas et al. 2021). Starch

reinforced with lignocellulosic fibers (cellulose and lignin) improved the mechanical properties (Yang et al. 2019), negatively impacting its biodegradability.

Incorporation of antimicrobial agents, such as neem-oil or oregano essential oil, slightly affected the biodegradation and disintegration of starch/PVA film. However, the incorporation of silver nanoparticles significantly reduced the biodegradability to 58% at 58 ± 2 °C after 45 days of composting, much lower than the starch/PVA film (68%). The starch/PVA/silver film lasts longer in the environment, due to the greater antimicrobial potential of silver than oregano and neem-oil (Cano et al. 2016).

Cassava-based simple bioplastic showed higher biodegradability than that of corn starch in burial test in soil for 14 days. Factors including the nature of starch, humidity, temperature, and enrichment of microorganisms showed an effect on the biodegradability of polymer cassava and corn starch (Zoungranan et al. 2020).

Hasan et al. (2020) observed that the addition of chitosan in higher content in the brown rice starch-based film significantly improved the tensile strength and thermal stability as well as decreased the biodegradability.

The application of acetyl tributyl citrate (ATBC) plasticizers in the PHBV matrix improved the biodegradation rate in the vermicomposting, freshwater biotope, and thermophilic composting. Further, in PHBV/ATBC with CaCO_3 or L-CNC bio-composite film, 57–60% are disintegrated in thermophilic composting after 30 days (Brdlík et al. 2022).

The main function of the plasticizers is to enhance the elasticity and mechanical strength of the bio-based polymers (Maraveas 2020). Generally, bio-based polymers are brittle because of strong intermolecular forces, such as dipole and hydrogen bonds. Plasticizers, such as glycerol and other polyols, are used to improve the toughness and flexibility of many polymers. The hydroxyl groups in glycerol can form strong hydrogen bonds with the polymers by breaking intermolecular hydrogen bonds in the polymer resulting in an increase in the system's molecular mobility (Özeren et al. 2021; Tarique et al. 2021).

Arrowroot starch (AS) plasticized with glycerol (15% w/w) showed 77.5% of weight loss when buried in soil compost after 20 days. Moreover, when increasing the glycerol concentration 15–45% (w/w), the biodegradability of the AS film was reduced. The addition of glycerol improves brittleness and flexibility. In contrast, it reduces the water vapor barrier properties (Tarique et al. 2021).

Water act as a plasticizer in gluten-based biopolymers, but it evaporates from the material depending on humidity and temperature. Therefore, another plasticizer, such as glycerol, is required to improve the processibility (Alonso-González et al. 2021; Özeren et al. 2021).

Gluten-based film (plasticized with glycerol), produced by various processing methods, including cast (hot-molded and cold and hot mixing), degraded completely within 50 days (Domenek et al. 2004).

Complete bulk degradation of PLGA in seawater and freshwater after 270 days, whereas PHB was degraded by 8% after 365 days, and PCL and PLA did not show any significant degradability (Bagheri et al. 2017). Table 2 summarizes the few recent findings on the biodegradability of various biopolymers.

Table 2 Biodegradability of different polymers

Biodegradable polymer	Conditions	Biodegradability (%) and observations	References
Cassava starch and corn starch	Burial tests in soil for 14 days	Cassava-based simple bioplastic showed higher biodegradability than corn starch Temperature, humidity, and microorganism enrichment influence biodegradability	Zounggran et al. (2020)
Cassava starch/polylactic acid films	Aerobic biodegradation of film (1 × 1 cm) under controlled composting conditions, 58 °C ± 2 °C for a month	65% of biodegradability in 32 days	del Rosario Salazar-Sánchez et al. (2019)
Acetylated or non-acetylated thermoplastic starch	Under composting condition: Material (2 mm pieces, i.e., 100 mg) under compost at 58 °C for 90 days	Biodegradation decreased with the degree of acetylation/substitution	Nevoralová et al. (2019)
	Under outdoor condition: highly acetylated material for 14 and 22 days	18–37% and 23–39% of weight loss after 14 and 22 days, respectively	
Thermoplastic starch (TPS)/polybutylene adipate-co-terephthalate (PBAT)/compatibilizer (either maleic anhydride [MA] or PBATg-MA)	Under compost conditions based on the emission of CO ₂ monitoring at 58 °C, 50% moisture content, with 10 mL/min air (CO ₂ -free) flow rate to provide aerobic conditions	Biodegradability of PBAT/TPS (40–60%) without any compatibilizer was 82–87% after 90 days, 72–74% in the presence of PBATg-MA	Dammak et al. (2020)
Thermoplastic starch/kraft lignin	Using soil microorganisms in the liquid biological medium at 30 °C for 10 days	Complete biodegradation before 10 days	de Freitas et al. (2021)
Tapioca starch/glycerol/polyvinyl alcohol (PVA) liquid/glycerol/bamboo fibers	According to ASTM G160, at a controlled burial location for 15 days	After 15 days, untreated, alkali-treated, and permanganate-treated samples lost about 5.44%, 5.97%, and 6.15% of their initial weight, respectively	Yusof et al. (2019)
Wheat gluten (WG)/shrimp shell powder	Accelerated weathering process under controlled conditions (UV, temperature, and moisture) for 120 h	WG, WG/shrimp shell powder, WG/calcinated shrimp shell powder composites showed 14.06%, 13.02%, and 4.29% of weight loss after 120 h	Thammahiwes et al. (2018)

(continued)

Table 2 (continued)

Biodegradable polymer	Conditions	Biodegradability (%) and observations	References
PLA/chitosan, PLA/CNC, PLA/gum	Aerobic composting condition using the thermophilic microflora already present in the compost at 58 ± 5 °C	Microbial colony formation (higher in PLA/Chitosan), contact angle confirmed the increase in hydrophilicity, and decrease in transparency	Kalita et al. (2019)
PLGA, PHB, PCL, PLA	Bulk degradation in seawater and fresh water at 25 °C under fluorescence light (16 h light and 8 h dark) for 1 year	Complete bulk degradation of PLGA in seawater and freshwater after 270 days PHB showed about 8.5% degradation after 1 year, while no significant biodegradability was reported with PCL and PLA	Bagheri et al. (2017)
PCL	Complete degradation under controlled conditions at 50 °C after 91 days in compost	Complete biodegradation (100%) after 91 days	Al Hosni et al. (2019)
Starch/polyhydroxyurethanes (PU) Starch/PU/cellulose nanocrystals	Film materials (44 cm) buried in the soil at a depth of 4–6 cm, 20–28 °C, 70% moisture content, and about 40% RH for up to 180 days	Starch/PU: mass losses about 66.4% after 60 days and 88% after 120 days Starch/PU/cellulose nanocrystals: about 44% after 60 days	Ghasemlou et al. (2022)
Starch/PVA, Starch/PVA/antimicrobial agents (neem-oil or oregano essential oil)	Under controlled composting at 58 ± 2 °C for 45 days	Essential oil slightly affects the biodegradation and disintegration behavior of starch/PVA film Biodegradability ranged from 68% to 114% after 45 days of composting	Cano et al. (2016)
Date palm leaf fibers reinforced PVA (polyvinyl alcohol)/corn starch hybrid composites	Soil burial method: composite material ($20 \times 5 \times 1$ mm) buried in farmland soil at a depth of 10 cm, 30 °C, 79–80% RH for 6 weeks	32% (PVA/corn starch containing palm leaf fiber, 40% wt) and 95% (PVA/corn starch) of weight loss after 6 weeks The incorporation of date palm leaf fiber into PVA/starch composite reduced biodegradability	Ray et al. (2021)
Brown rice starch/chitosan plasticized by palm oil	Material (2.5×2.5 cm) buried in compost media at 10 cm depth for 20 days	Highest biodegradation rate in film contains 70% (w/w) of starch	Hasan et al. (2020)

(continued)

Table 2 (continued)

Biodegradable polymer	Conditions	Biodegradability (%) and observations	References
Gluten/glycerol (plasticized gluten) (cast, hot-molded, and cold and hot mixed gluten materials)	Mineralization of material in aerobic fermentation (liquid medium, Modified Sturm Test) Samples (0.5 g) buried in farmland soil 20 °C	85% mineralization after 36 days in aerobic fermentation (liquid medium, Modified Sturm Test) 100% weight loss after 50 days in farmland soil	Domenek et al. (2004)
PHBV/acetyl tributyl citrate (ATBC) with CaCO ₃ or lignin-coated cellulose nanocrystals (L-CNC)	Thermophilic composting in accordance with ISO 14855-1 standard (mineralization: 10 g [1 × 1 mm], 58 °C, in 150 g compost for 3 months; biodegradation: films 100 × 35 mm, 58 °C, 50% humidity, for 30 days), vermicomposting (films 100 × 35 mm, 21 °C, 81% MC, for 30 days), and freshwater biotope (films 100 × 35 mm, 27 °C, for 30 days)	Highest biodegradation rate in thermophilic composting (>90% after 90 days) Degree of disintegration thermophilic composting in PHBV/ATBC with CaCO ₃ or L-CNC (57–60% after 30 days) Application of ATBC increased the biodegradability in all medium	Brdlík et al. (2022)
Arrowroot starch (AS) films using glycerol at 15, 30, and 45% w/w	Soil burial degradation: 20 × 20 mm film was buried in the depth of 100 mm soil compost	In AS film, addition of glycerol reduced the tensile strength (16.48–1.95 MPa) and modulus (1258.9–36.08 MPa) and increased the elongation at break (2.49–57.33%) and water vapor permeability (to 10.83 g·s ⁻¹ m ⁻¹ Pa ⁻¹) Weight loss in AS and AS/glycerol (15% w/w) were 74.4% and 77.5%, respectively, after 20 days	Tarique et al. (2021)

Conclusion

Biodegradable polymers have been widely used as a substitute for petroleum-based polymers due to their cost-effectiveness, availability, nontoxicity, biocompatibility, biodegradability, and feasibility of producing films alone or in combination with other polymeric matrixes. Many polymers, polysaccharides, proteins, and other

microbial polyesters have been used in food, packaging, agriculture, biomedical, environmental remediation, and many other fields.

Natural polymers have a poor mechanical, thermal, barrier, and other functional properties. As a result of these constraints, natural polymers undergo various physical and chemical modifications, including blending with other polymers or incorporating with fillers, additives, and nanoparticles. These modification processes may have an influence on the overall biodegradability. Therefore, it is essential to assess the biodegradability of polymer material. Various studies on the biodegradability of different biopolymers suggest that biodegradability can be altered by the process method, combinations of polymer, structure, and environmental conditions. Since biopolymers are treated with additives, including NPs, plasticizers, and other chemicals, the migration behavior of these materials should be extensively studied.

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Biopolymer Waste Management

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Reuse, Recycling, and Disposal

Azeem Intisar, Arooj Ramzan, Mateen Hedar, Nazim Hussain, and Muhammad Bilal

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Abstract

Because of their significant contribution in various products present in modern society, biopolymers are regarded as a diverse class of materials. The designing and marketing of renewable and suitable biopolymers have attained a serious development in recent decades. However, on the other side, their frequent utilization and waste pose a number of environmental risks including pollution, health, and depletion of nonrenewable natural resources. Although most of the

A. Intisar · A. Ramzan · M. Hedar
School of Chemistry, University of the Punjab, Lahore, Pakistan

N. Hussain (✉)
Center for Applied Molecular Biology (CAMB), University of the Punjab, Lahore, Pakistan
e-mail: nazim.camb@pu.edu.pk

M. Bilal (✉)
Institute of Chemical Technology and Engineering, Faculty of Chemical Technology, Poznan
University of Technology, Poznan, Poland
e-mail: muhammad.bilal@put.poznan.pl

biopolymers offer some kind of waste management solution, there are limitations to their efficacy in reducing vulnerabilities associated with them. Proper waste management strategy is obviously a definite requirement to protect the environment from their harmful impacts. In this chapter, different strategies that have been successfully applied for the reuse, recycling, and disposal of biopolymer waste are discussed. Moreover, the pros and cons of methods to treat this waste are also discussed. The applicability of biopolymer waste treatment at domestic and industrial level has also been elaborated.

Keywords

Biopolymers · Waste management · Recycling · Reuse · Disposal

Introduction

Biopolymers are organic molecules that are found in natural sources. The word biopolymer is derived from two Greek terms “bio” and “polymer.” These words reflect the life and the natural environment. These are larger macromolecules composed of multiple repeating units (Ezeoha and Ezenwanne 2013). Biopolymers are made up of a wide variety of molecules, each of which has a unique chemical structure. Some of these include proteins, polysaccharides, polyamides, bioplastics, polyesters, etc. They are biodegradable and biocompatible, which makes them useful in a variety of uses like packaging materials, emulsions, edible films, drug transport materials, wound healing, and tissue scaffolds, medical implants, and dressings in the pharmaceutical industries (Baranwal et al. 2022). Besides, they are quite abundant in nature and have a wide variety of applications; yet, they have also developed into one of the most serious wastes produced by industrial and agricultural processes, which contributes to environmental contamination. (Bayón et al. 2018).

The term waste was examined and redefined as an emergent property of a substance. When a substance is not employed to the fullest extent, it is said to be a waste. Under this concept, any method can be utilized for the conversion of waste to get rid of this quality label, and it becomes abundantly clear that there is a need for a comprehensive approach to the management of resources and waste (Dijkema et al. 2000). The ever-increasing human population and the spread of urbanization have both contributed to the constantly increasing quantity of waste. Manufacturing processes, industrial activities, and municipal solid waste (MSW) lead to the formation of waste products (Kan 2009). MSW refers to waste that includes nondurable products, durable products, food scraps, packages, yard clippings, and various inorganic wastes that arise from industrial, commercial, and residential sources.

The majority of biopolymer waste originates from the food service and healthcare industries. Bottles, utensils, cups, plates, trays, straws, towels, toothpicks, etc. are examples of food service items, whereas medical tubing, gloves, blood pressure cuffs, face masks, bandages, adhesive tape, waste bags, sheets, syringes, enema tips and bags, intravenous liquid bags and tips, etc. are examples of healthcare products.

Waste from hospitals and other medical facilities poses a threat to public hygiene and must undergo special processing before being disposed of. Incineration is the preferred method for disposing the vast majority of hazardous wastes that are produced in the healthcare industry, and it is still regularly utilized. Additional sources of waste material include forestry, agricultural, automotive, and construction products that are manufactured from biopolymers. Residues from post-industrial processes are another key cause of waste biopolymers (Niaounakis 2013a). Recently, about 140 million tonnes per year of biopolymers are manufactured across the globe with the vast majority originating from resources derived from petroleum. They have broad applicability as packaging materials in the food, pharmaceutical, cosmetic, detergent, and chemical industries (Shah et al. 2008). Approximately 30% of this manufacturing is used for packaging purposes, with around 90% of that being used for food packaging. Their use is constantly growing, at a yearly rate of 12%. This means that industrial wastes bring a significant proportion of these chemicals into the environment. Plastics have acquired more public and media attention than any other type of solid waste because of their increased visibility in litter (Siracusa 2019).

The waste management has emerged as one of the most serious environmental challenges of the world. The increase in the rates of solid waste generation has been attributed to human activities, as well as modifications in lifestyle and consumption patterns. The method of recovering resources from waste is another part of waste management. The management of waste may involve the treatment of solid, liquid, gaseous, or radioactive materials, each of which requires a unique set of procedures and areas of expertise (Dijkema et al. 2000; Kan 2009). Sewage disposal, wastewater treatment, and biological and chemical processing comprise liquid waste management. The waste management strategies of developing versus developed nations, rural versus urban areas, and residential versus industrial areas may vary. Recycling generally refers to the extraction of resources from waste, as well as the recovery or reuse of the extracted material. There are a variety of ways to recycle waste material; the raw materials can be recovered and reprocessed, or its heat can be transformed into energy (Demirbas 2011). Figure 1 depicts the basic components of waste management: collection, transportation, recovery, recycling or disposal, and analysis of waste substances. A typical system of waste management consists of residue collection, transportation, treatment, processing, and finally disposal.

The residues of biopolymer residues can be gathered from producers and end users. It is preferable to collect waste biopolymers from industry because they produce them in vast quantities. Some organizations that specialized in recovering waste biopolymers from major gatherings such as sports and concerts may choose to collect from the end users directly. When these events take place, huge volumes of biopolymer waste are generated as a result of the use of bottles, cups, and fast-food items. A majority of these wastes are disposed of in one of two ways: either they are placed in trashcans and sent to landfills or, at best, they are taken to a waste treatment company (Niaounakis 2013a).

The goal to manage waste is creating a sanitary living conditions, minimizing the number of chemicals entering or leaving the community, and promoting the reuse of substances in the community (Demirbas 2011). The huge amounts of waste cannot



Fig. 1 Waste management process

be removed. Yet, the effect on the environment can be mitigated by finding more environmentally friendly uses of it. That's what it refers to as the waste hierarchy (Batayneh et al. 2007). There are three levels of the waste hierarchy, which categorize waste management solutions in terms of waste minimization: reduce, reuse, and recycle. Reducing, reusing, recycling, composting, incinerating, and dumping are the six disposal choices in descending order of environmental impact. The waste hierarchy serves as the basis of the majority of waste reduction approaches. The objective of the waste hierarchy is to extract the maximum benefit from products while generating the least quantity of waste (Siddique et al. 2008). The scope of this chapter is to present a brief overview of waste management of biopolymers including possible ways of its reuse, methods of physical and chemical recycling, and known techniques of their disposal.

Reuse of Biopolymers

Reuse is a typical approach that can be implemented to discarded or used polymer items. It is preferable to reuse biopolymers because of less energy consumption and use of cheaper resources. Defective preforms or molds, as well as worn articles, are reprocessed in their current state without the chemical composition of the

biopolymer being altered in any way. Biopolymers can be reused in the following ways (Niaounakis 2013b):

- The immediate recovery of scrap material during the molding process
- Mixing the polymer materials with other polymer materials, frequently in association with compatibilizers, to produce new materials
- Combining with a variety of chemicals, including reinforcing agents and antioxidants
- The alteration of the structure of the polymer so that it can regain its original qualities

In the process of recuperation, industrial wastes are ground up, combined with virgin material, and then immediately injected into the processing machines. This is one of the most common methods for recycling scrap materials from the manufacturing industry. The treatment through heat is needed for the process of agglomeration, but the biopolymers should not be changed thermally. If this happens, the biopolymers will degrade chemically, release gases that are mostly harmful, and make wastes unusable for the technological reuse that was intended for them (Vilaplana and Karlsson 2008). The primary technical challenges that need to be taken into account to produce a material with attributes that are comparable to those of the virgin one are (i) modifying the ratio of recycled materials in the blend and (ii) optimizing the processing technique (K.G., P.M.G.C. *n.d.*).

The treatment of biopolymers wastes results in products that, on average, have lower-quality features than the materials from which they were originally derived. This is because of differences in chemical structure that were brought about by earlier phases of degradation. The procedure of restabilization is known for its role in shielding recycled substances from the thermomechanical deterioration that occurs during reprocessing and in strengthening the long-term durability of the materials through reuse (Södergård and Näsman 1994). It cannot efficiently recover the damaged material, but it can stop further chemical changes that may be accelerated by the oxidative compounds that are found in its composition or by humidity. It has been observed that certain additives, such as catalyst deactivators, can stabilize PLAs in the melt. 1,4-Diaminoanthraquinone (McNeill and Leiper 1985a), benzoyl peroxide (Södergård and Näsman 1996), and 2,4,6-cycloheptatrien-1-one,2-hydroxy (McNeill and Leiper 1985b), natural and biodegradable acids, are the examples of such additives. In order to delay the biopolymer hydrolysis, additives like carbodiimide substances are often used (Hartmann 1999).

The most prevalent and known method for improving the qualities of single-stream biopolymers wastes is the blending of recycled polymers with virgin materials (Imre and Pukánszky 2013). Recycling technology has been created by Sharp, which allows for the repeated recovery of plastic from used consumer devices and the reuse of the material in the construction of new consumer devices. In 2009, Sharp (JP2009161655) published a method for reusing waste bio-based thermoplastic resins. The method involves melting or heating a mixture of the waste thermoplastic resin and at least one polymer chosen from the group consisting of vinyl-based

polymer, aliphatic polyester, and a styrene-based polymer. In 2011, DU PONT (WO2011146562) revealed a technique for the production of a fiber that contained 0.1–99.9 weight percent of recycled poly(trimethylene terephthalate) (PTT), with the range of 5–10 weight percent being preferred (Niaounakis 2013b; Teaca et al. 2018).

The composition of the recyclates can also be chemically modified, which is yet another method for efficiently upgrading the functionalities of recycled materials. During melt reprocessing, the use of various substances, like substances having reactive functional groups, radical generators, or chain extenders can be able to induce branched and cross-linked reactions or enhance the molecular mass of deteriorated biopolymer chains derived from wastes (Hu 2014). This type of treatment will result in the increase of weight of polymer chain, as will the improvement in their mechanical and rheological aspects. Upgrading of deteriorated bio-based aromatic polyesters achieved through the application of chemical modification has proved successful (Arbenz and Averous 2015).

The reusability of polymeric materials can be evaluated using multiple processing methods, and this can also be employed to examine the thermomechanical degradation that occurs during physical recycling. The study of alterations in structure and morphology brought on by successive processing steps provides valuable data that may be used to optimize the processing parameters during physical recycling to prevent further degradation of materials (Pillin et al. 2008). The selection of appropriate conditions for processing and the subsequent addition of stabilizing agents and many other chemicals is one example of this method. The macroscopic features and molecular structure of biopolymers are altered when they are subjected to several processing steps. A reduction in the weight of biopolymer chains is caused by chain scission. This in turn causes a reduction in viscosity, a rise in crystallinity, and a variation in the mechanical characteristics of the material. This leads to a gradual increase in the brittleness of the material that has been reprocessed (Hamad et al. 2011).

Recycling of Biopolymers

The market for biopolymers is expanding quickly as a result of the public's growing concern for ecological sustainability (Rajan et al. 2019). Researchers have shown a great deal of interest in this relatively new area to explore the usage of biopolymers as biofuels (Ma and Sahai 2013). Additionally, bio-based products can be progressively used in medical applications such as surgery and therapy, as well as drug delivery systems, and find its potential use in nanomedicines (Kabir et al. 2020). Despite the rapid expansion of biopolymers, their waste streams are still lower and dispersed. On the other side, stress is laid on the recycling of these biopolymers (Cornell 2007). The proper design for waste management of biopolymers is necessary both for the environment and for the circular economy by utilizing these wastes in an appropriate way. Different strategies are used to recycle waste biopolymer. Recycling biopolymers is essential for cutting down on the amount of renewable

resources needed to make the equivalent monomers to produce new polymers using them (Soroudi and Jakubowicz 2013).

Biopolymer recycling is a controversial subject. The merits and demerits of biopolymer recycling are the topics of the current discussion. The critics claimed that recycling does not take advantage of the biodegradability of biopolymers. In addition, biodegradable polymers represent a significant issue when they enter systems for traditional plastics recycling or decomposition of green waste (Cornell 2007). The main techniques for the recycling of biopolymers are:

- Physical or mechanical recycling
- Chemical recycling
- Enzymatic De-polymerization

Mechanical Recycling

In mechanical recycling, discarded polymers are put through mechanical processes like grinding (Åkesson et al. 2016), crushing, and extrusion (Resch-Fauster et al. 2017). Waste material is subjected to washing and drying prior to mechanical method. The polymers are kept intact during mechanical recycling, allowing for numerous uses of the polymers in the formation of the same products or for their applicability in different fields (Niaounakis 2019). These processes of mechanical methods are illustrated in Fig. 2.

Cellulose nanofibers (CNF) are progressively gaining attention because of its renewability as compared to synthetic polymers. CNF can be isolated from the waste

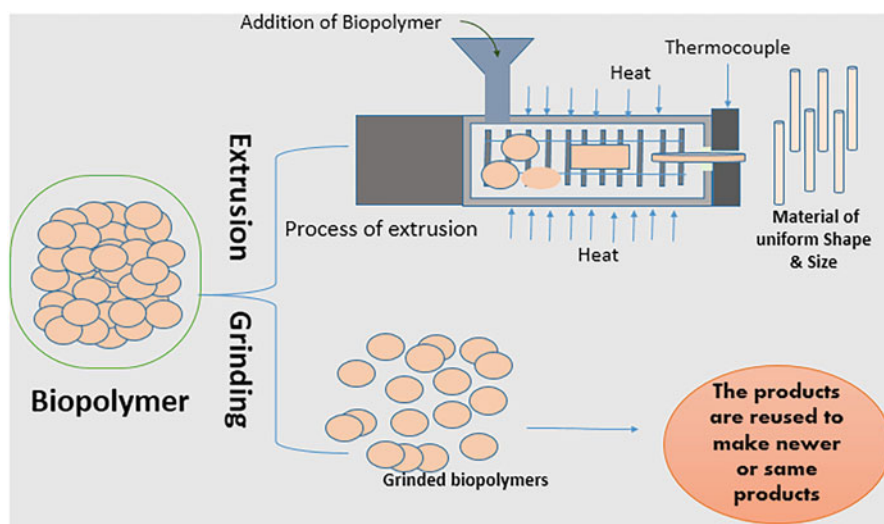


Fig. 2 Different mechanical techniques used for the recycling of biopolymers

material of industry which uses cellulose-containing materials as raw materials, e.g., paper industry. However, it will be possible to scale up from the laboratory to the commercial applications if its production from waste material is cost-effective to compete with cheap synthetic polymers. Josset Sébastien et al. used the process of grinding to study the direct fibrillation of cellulose-containing matrix. The basic purpose of this study is to find the cost-effectiveness of this process. Wheat straw and secondhand newspaper which are regarded as waste material are used as raw material. Bleached wood pulp was also used for comparison. The mechanical characteristics and specific surface areas of the CNF were studied and compared. The greatest values for the characteristics of the bleached wood pulp were already attained with little energy cost. The various characteristics of CNF isolated from waste materials might reach values close to their maximums with energy input as low as about 5 kWh/kg and high-pressure homogenization required 10 kWh/kg. (Josset et al. 2014).

Biopolymer waste is passed through a series of mechanical operations, such as crushing, grinding, and melting, as part of the mechanical recycling of biopolymers. In some cases, these procedures are along with drying or washing. Because the polymer materials are not damaged during the process of mechanical recycling, it is possible to reuse the polymers several times in the similar or comparable material. Mechanical recycling is a promising approach for recycling typical polymers; however, it cannot be effectively applied to the recycling of biopolymers. Aliphatic polyesters make up the vast majority of commercially available biodegradable biopolymers. These include biopolymers like polylactic acids (PLAs), polyhydroxyalkanoates (PHAs), and polyglycolide (PGA). PHAs are members of one of the families of commercially available polymers that exhibit the highest degree of temperature sensitivity, whereas PGA and PLA are both extremely prone to thermal decomposition, which results in discoloration and a loss of molecular weight and is caused by heating the material. Hygroscopicity, sticking at a higher temperature, and thermal destabilization are some of the other issues (Niaounakis 2019). The recycling potential of PGA and PHA is still practically nonexistent except for PLA. To avoid hydrolysis of the molecular chains in the plasticization phase of the PLA reprocessing, it is essential that the material being recycled be as dry as feasible. However, PLA being a hygroscopic material, drying it effectively might be challenging. Additionally, the existence of paper contaminants that contain a significant proportion of moisture renders the process of drying more complicated (Hiebel et al. 2017). Furthermore, the low temperature range (55–60 degrees Celsius) at which PLA seems to become sticky, combined with its relatively large time of crystallization, makes it difficult for drying or crystallizing using typical drying or crystallization systems. In a similar manner, plasticized starch is susceptible to the process of hydrolysis while in use, and it can't be reprocessed again for a similar purpose. The recrystallization, grinding, and drying of the biodegradable wastes are some of the suggested solutions to the difficulties listed above that are experienced in the physical recycling of chosen aliphatic polyesters (Niaounakis 2019).

Chemical Recycling

The term chemical recycling refers to the various chemical methods that break down biopolymers waste into monomers or oligomers or directly into some other valuable materials. The techniques for chemical recycling are varied and numerous, and depending on the type of polymer being recycled, each approach will have both benefits and drawbacks associated with it (Payne et al. 2019; Hopewell et al. 2009). In chemical recycling method, different reactions are carried out between reagents and biopolymers to obtain the subunits or monomers which are utilized to make new biopolymers to make different products (Lamberti et al. 2020a). In chemical method, dry heat is also used to break the bonds in material to depolymerize it (Niaounakis 2013c). The products of depolymerization are again polymerized into newer compounds. This method saves the material source, and it is beneficial for circular economy. Mostly solvolysis (Thiele et al. 2004) and depolymerization (Ponomarev 2018) methods are used in chemical recycling. These Chemical methods used for recycling are illustrated in Fig. 3.

Agaricus bisporus is an edible mushroom which is mostly used in the United States. A majority of the waste generated during mushroom cultivation and reap are stubbles and mushrooms with erratic shapes and sizes. The waste is 20% of the total yield. As a result, there is about 50,000 metric tons of garbage produced per year that cannot be used for viable purposes. The heaps of these wastes can be hazardous to the environment because of its decomposition and high volumes. However, the waste of this mushroom contains large amounts of chitinous biopolymers which find potential applications in bioinsecticide (Bhaskara Reddy et al. 1999), antimicrobial agent (Roller and Covill 1999), and it is also used to coat materials (Yu et al. 2020) and for water treatment methods. Wu, Tao, et al. extracted the chitinous material from the waste of these mushrooms and also determined glucosamine.

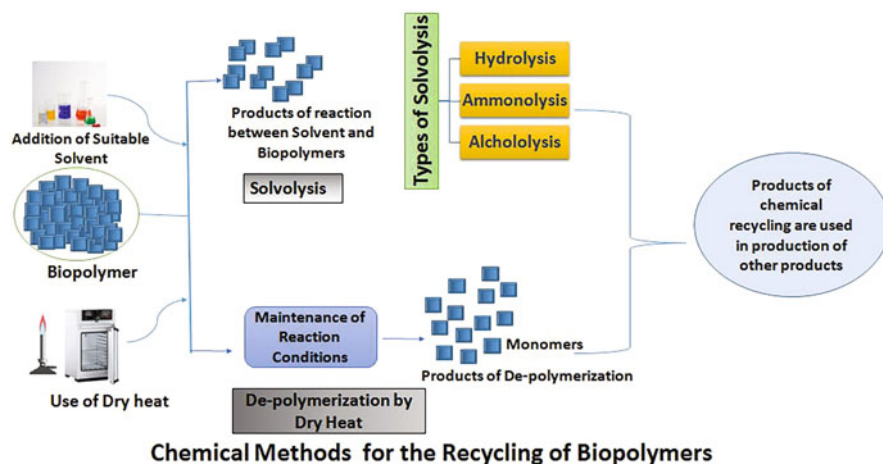


Fig. 3 Strategies used in chemical recycling of biopolymers

The acid and alkali treatment method was used to extract chitin from waste, and it was hydrolyzed to determine glucosamine. During postharvest storing, the chitin material in mushroom stalks increased to 19.02% dry weight (DW) which can be effectively removed. Given the type and volume of waste generated by mushroom cultivators each year, the suggested method might produce around 1000 metric tons of fungal chitin yearly. These materials obtained from waste can be effectively used as biopesticide, antimicrobial agent, and feed additive to regulate the growth of plants. This study was the initial step to change mushroom waste into a useful product (Wu et al. 2004).

The recycling of biopolymers through chemical methods is related to the restoration of usable chemical constituent such as any monomer or oligomer from the waste material. Recycling biopolymers is mostly done to preserve the material resources, reuse the recovered monomers, or make new polymers. The main chemical recycling techniques are (Niaounakis 2013d, 2019):

- Depolymerization by dry heat
- Hydrolysis
- Alcoholysis

Depolymerization by Dry Heat

It is a process that involves the conversion of polymers like PLA to their cyclic dimers or lactide form by subjecting them to temperatures higher than their transition temperatures in catalyst's presence. The depolymerization of PLA into its cyclic dimer via heating and reduced pressure is an old method that has been the subject of extensive research (Nishida et al. 2003; Fan et al. 2004). The method of depolymerization of PLA of high molecular weight by dry heat was reported by UHDE INVENTA FISCHER (EP2559725 A1) in 2013. The method has a limited polymeric production and is quite slow because of relatively low end-group composition; therefore, the reaction rate must be enhanced by increasing the temperature and catalytic concentration. According to a 1994 report by DU PONT (US5342969), thermal decomposition of PLA happens at an elevated temp, various side reactions take place, and it is hard to achieve a highly pure product. Furthermore, the racemization of the lactide happens when PLA is depolymerized by dry heat above its T_m , regardless of the PLA's molecular weight (Niaounakis 2019). Utilizing specialized catalysts at temperatures higher than T_m allows one to overcome the issues that were discussed earlier. The depolymerization catalysts are responsible for temperature reduction throughout the depolymerization process. The rate of thermal disintegration of PLA is accelerated by the use of the depolymerization catalyst, and the molecular weight of PLA immediately begins to decrease. One example of a catalyst is magnesium oxide that can be used to depolymerize PLA, whereas strontium oxide, calcium oxide, and barium oxide are some examples of additional alkaline earth metal oxides that may potentially be applied. Sn(II) 2-ethylhexanoate can also be utilized as a polymerization catalyst, and aluminum hydroxide can be employed as a flame retarder (Niaounakis 2019).

Hydrolysis

Hydrolysis is a chemical technique that has showed a great deal of potential for recycling biopolymers through depolymerization into its monomer subunits. Neutral hydrolysis, acidic hydrolysis, and alkaline hydrolysis are the three main kinds of hydrolysis which have been investigated in depth. In neutral hydrolysis, water or steam is used, heated to high temperatures (between 200 and 399 degrees Celsius), and subjected to high pressures (between 1 and 4 MPa). When the reaction takes place at temperatures that are quite close to the melting point of polymers and high-pressure mechanisms are applied, then high depolymerization rates can be achieved (Sinha et al. 2010). In acid hydrolysis, in addition to other mineral acids such phosphoric or nitric acid, concentrated sulfuric acid is the primary acid used. During the process of alkaline hydrolysis, an aqueous alkaline solution of sodium hydroxide or potassium hydroxide that has a concentration ranging from 4% to 20% by weight is used (Grigore 2017).

The hydrolysis of biopolymers relates primarily to the depolymerization of aliphatic polyesters in an aqueous medium at high temperatures and pressure in acid catalyst's presence. Lactic acid (LA) is produced through the hydrolysis of polylactic acid (Niaounakis 2019). Within two hours at temperatures between 160 and 180 degrees Celsius, PLAs undergo hydrolysis to produce LA at a conversion rate of 95 percent. Third-order kinetics is followed by the hydrolytic deterioration of PLA because the hydrolysis rate is dependent on the amount of water, biopolymer linkages, and acid-hydrolyzed byproducts (Piemonte and Gironi 2013). It is essential to remember that PLA hydrolysis is an autocatalytic process. This indicates that carboxyl groups that are formed on every hydrolytic cleavage will catalyze the reaction further. If the medium used for hydrolysis is basic, then the process of ester cleavage is arbitrary; however, in acidic medium, the process is chained unzipping. Rate constant, diffusion coefficient of chain fragments, percentage of absorbed water, solubility of byproducts, temperature, crystallinity, and pH of the PLA are the factors that affect hydrolytic degradation (Schliecker et al. 2003). The recovery of LA is an excellent illustration of the potential provided by chemical recycling method. It has been predicted that LA production by corn fermentation requires approximately 55 MJ energy per kg of lactic acid produced, whereas the production of LA through PLA hydrolysis requires a significantly lower amount of energy, approximately 14 MJ of energy per kg of LA produced (Piemonte et al. 2013).

Alcoholysis

Alcoholysis is a chemical process in which alcohol acts as the nucleophile. In the process of chemically recycling polyesters, a transesterification reaction takes place in which the alcoholic group breaks the ester linkages. Polymer is then depolymerized into its monomeric units or into materials with additional value (Lamberti et al. 2020b). For instance, valuable products can be produced from PLA by depolymerizing it through the process of alcoholysis. During a transesterification reaction, a wide range of alcohol groups are employed to target the

ester linkages of PLA, which then results in the production of lactate esters. In addition to this, a transesterification catalyst is needed to achieve adequate depolymerization under moderate reaction parameters (Román-Ramírez et al. 2018). Depolymerizing PLA waste with methanol, ethanol, propanol, or other solvents will result in the production of methyl lactate, ethyl lactate, and propyl lactate, each of which is a useful chemical in the manufacturing industry (Bowmer et al. 1998).

DU PONT (US5264614 A) reported a procedure for recycling aliphatic hydroxyl carboxylic acid-derived polyester wastes in 1993. The method involves heating of the waste in the presence of lower alkyl alcohols, i.e., CLEC6 alcohol or in H_2O , under high pressure in order to make the polyester soluble; the heating is carried out in such a way as to prevent deterioration of polyesters and by recovering polymers after the solution has been cooled (Niaounakis 2013d). In 2009, TEIJIN FIBERS LTD (P2009029757) reported a technique for the depolymerization of PLA. This approach involves the use of a catalyst that is simple to handle. Alcoholysis of PLA (such as Lacty[®] 9010, manufactured by Shimadzu) with methanol at temperatures ranging from 60 to 100 degrees Celsius in the vicinity of catalyst of zinc-containing compounds that is made of $ZnCO_3$ or zinc carboxylates is the depolymerization process. It was possible to obtain a sufficient amount of methyl lactate. PLA is derived from resources used in agriculture and fishing as well as containers used for food packaging and materials used in civil engineering and building (Niaounakis 2015).

Enzymatic Depolymerization

A majority of the chemical and thermal recycling processes described above require a significant amount of energy input, produce a low quantity of monomers in a short time, and eliminate additives and catalysts that were employed in the alcoholysis, hydrolysis, and depolymerization steps (Tsuji et al. 2003). The enzymatic depolymerization of biopolymers is an emerging and potentially useful recycling technology. The primary objective of this method is to reuse the products formed as a result of enzymatic degradation. This is what differentiates it from the disposal. The ability to polymerize and depolymerize a substance in an enzyme-catalyzed reaction is one of the benefits of using an enzyme, particularly hydrolases. The action of a hydrolase enzyme is used to cleave biodegradable biopolymers that include enzymatically hydrolyzable components such as esters. This step is necessary before biodegradable biopolymers may be further processed. To develop the enzymatic recycling of biodegradable biopolymers, like PHB, poly(butyl acrylate) (PBA), PLA, polycaprolactone (PCL), and poly(butylene adipate co-succinate) (PBAS), the enzymatic depolymerization and re-polymerization processes were executed. In a solution of organic solvents that also contained a minute quantity of water, lipase was used to degrade them, which resulted in the production of cyclic oligomer. This oligomer was easily polymerized again in great amount by the same catalyst, which resulted in the production of polyester with a molecular weight that was either equivalent to or

higher than that of the original polymer (Kobayashi et al. 2000; Takamoto et al. 2001; Takahashi et al. 2004; Matsumura 2002).

Keio University in Japan is responsible for the vast majority of the work that has been done thus far in the field of enzymatic recycling of biopolymers. A technique for manufacturing trimethylene carbonate (TMC) was disclosed in 2002 by UNIV KEIO (P2002017384). This approach involves selectively transforming a poly(trimethyl carbonate) (PTMC) into a polymerizable polymer in the presence of hydrolase. The depolymerizing hydrolase was successfully retrieved and put to further use without suffering any apparent loss of activity. The lipase that is developed from *Candida antarctica* (Novozym® 435) is the most commonly used. After adding the lipase to the chloroform, it was filtered to separate the components. Through the use of an evaporator, the solvent was extracted from the filtrate and concentrated. The silica gel column chromatography method was used to obtain 75 percent of the expected yield of trimethylene carbonate. Re-polymerization of PTMC that has been converted into monomer can occur in a selective manner. The recycling of PTMC by using an enzyme was reported by Matsumura et al. Using lipase from *Candida antarctica* in acetonitrile at a temperature of 70 degrees Celsius, the enzymatic transformation of PTMC with M_n of around 3000 to approximately 48,000 yielded the comparable cyclic monomer (TMC) yielding up to 80 percent (Matsumura et al. 2001).

A hydrolase was used in a depolymerizing process that was published in 2002 by UNIV KEIO (JP2002017385) to synthesize dicalpolactone. This method involved the selective transformation of PCL to monomer that can be polymerized again. By applying this process of depolymerization, the dicalpolactone can be produced in large quantities, and it is then possible to re-polymerize the dicalpolactone in the presence of a hydrolase, like lipase that is derived from *Candida antarctica*. Because enzyme activity is rarely diminished, the hydrolase that was used for depolymerization can be recovered and reused on multiple occasions (Ebata et al. 2000). To enhance intramolecular cyclization, the depolymerization of PCL by using enzymes into dicalpolactone demands high volumes of organic solvent like toluene solution (0.2%) (Kondo et al. 2002).

Disposal of Waste Biopolymers

Disposal of waste biopolymer is the last option for the end of the life products. Various techniques are used to dispose of the waste biopolymers which are illustrated in Fig. 4. Synthetic biopolymers cause more hazards to the environment as compared to bio-based material. The disposal mechanisms used for these wastes are not so efficient and eco-friendly. These waste materials cause pollutions and are hazardous to human being. Moreover, some biopolymer wastes are biodegradable, while others are not. Ecological conditions are deteriorated by disposing wastes in an inappropriate way (Kuppens et al. 2010). Therefore, different strategies are employed to dispose waste materials into the environment.

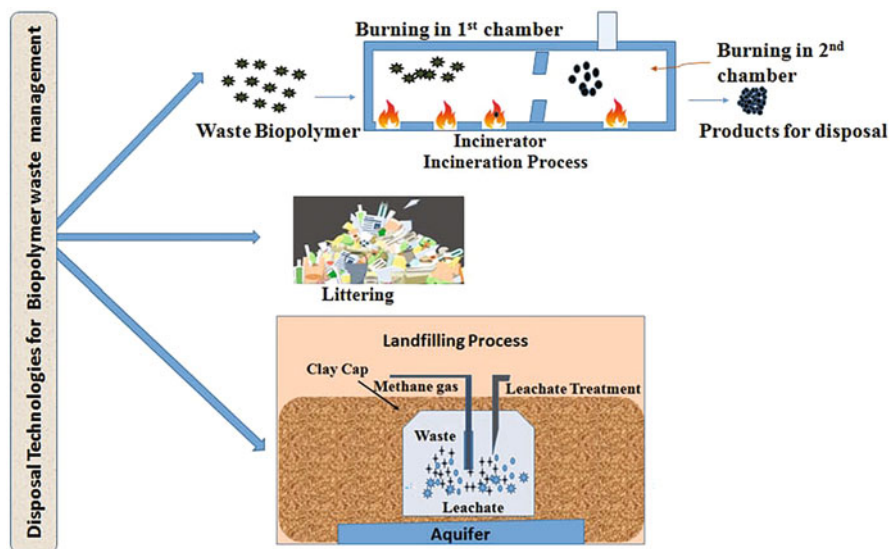


Fig. 4 Different strategies used for the disposal of biopolymers

Over the past ten years, the use of biodegradable natural polymers has increased in the United States, and it is anticipated that this trend will continue in the years to come. Few researchers have looked at how consumers actually use and discard biopolymers, despite the fact that some have been conducted to evaluate their environmental impacts. Meeks, Diana, et al. accessed and studied the data where biopolymers are used and their disposal. Audits of nearby grocery stores and semi-structured interviews with compostable biopolymer users in four separate food service types (restaurants, catering businesses, limited food premises, and outdoor recreation concessionary) were done to determine where compostable biopolymers are still being used. According to research, commercial food settings are where customers are most likely to come into contact with compostable biopolymers. Composability of biodegradable biopolymers was not the only consideration when deciding whether to buy them; other considerations included their presumed sustainability (Meeks et al. 2015). Accessibility to the infrastructure for composting and the general growth of the compost industry were the two most significant factors for all companies when making decisions about how to dispose of compostable biopolymers (Yates and Barlow 2013). The decision to compost biomaterials may even have the effect of diverting food waste from landfills, minimizing the environmental effects of both food scraps and the dumping of natural polymers (Levis and Barlaz 2011). Environmental impact studies will only be able to produce significant results that can guide best practices for municipal garbage management if they are conducted with a full knowledge about the real-world use. Sometimes the disposed waste material is valorized to form useful products. The development of foodstuff packaging materials using biopolymer derived from food processing wastes is seen to be a sustainable option to improve resource efficacy, and moreover, it lowers the

environmental issues caused by the disposal of packaging waste. Proteins exhibit considerable potential because of their richness in nature and inexpensive availability. Gelatin obtained from different sources, including fish, bovine, and pig, can be used to make active packaging. Gelatins find its use as bioactive component carriers for foodstuff packing in addition to medicinal uses. The shelf-life of food can be extended and food losses decreased by adding natural antioxidants and antibacterial agents in the formulations that create films (Etxabide et al. 2017).

The main global concern is the adverse effects of waste disposal on the environment because contamination and harmful compounds present in waste can migrate into soil and water bodies and through seepage can contaminate and pollute the groundwater as well. As polymers are complex structure made by the reaction of many repeating units called monomers, their degradation is not easy. A large amount of synthetic and natural rubbers is used in day-to-day life for various purposes (Shah et al. 2013). Their disposal on land causes harmful impact to the environment. Additionally, because there are fewer landfills available for the safe disposal of rubber debris, challenges with water and land pollution are being created by improper disposal of waste. One of the primary causes of climate change is the tremendous amount of energy released in the form of heat and smoke produced when rubber scrap is burned (Stevenson et al. 2008). Recovering or reclamation of rubber is a more advantageous technique of disposing waste rubber products because it not only conserves our finite supply of fossil feedstock but also preserves environmental quality (Adhikari et al. 2000). To save the environment, disposal regulation standards must be set and followed by the local government, industries, and domestic people. Proper treatment of waste is necessary before disposing them on open places (Ustra and Elis 2018).

Landfill

Biopolymer waste is disposed in landfills. It generates gases, and their uncontrolled gas release enhances the biological activity in organic matrix present in waste. If proper method is used, the gases released during landfill can be collected and used to convert energy to incinerate the waste material. Methane is usually released during landfill, but its amount is higher in the early stages, and later on a decline in its amount is observed. With the decline in methane content, the incineration process becomes difficult and expensive. Inert material is more preferred in landfill because they don't participate in the creation or emission of carbon dioxide or methane in an uncontrolled manner. Biopolymer landfills are generally considered as the least preferable alternative for dumping because they produce no benefits while simultaneously increasing emissions of greenhouse gases (Endres and Siebert-Raths 2011). Kendall, Alissa et al. studies the life cycle assessment (LCA) to investigate a possible path for generating PHB from the cellulosic portion of organic waste from material recovery facilities that otherwise would have been dumped in a landfill. This limitation applies not just to landfill activities, but also to any long-term processes related to product disposal. Increased variation in CO₂ emission estimations for PHB results from taking into account fluctuations in landfill gas

output, with values ranging from 1.7 to 6.3 kg carbon dioxide equivalent/kg PHB. The analysis of the evolving fate of carbon in MRF residuals revealed a prevailing weakness in LCA procedure: landfill simulation tools were not so accurate, and there was no agreement or a set of guidelines for choosing an analytical time horizon for taking into account landfill gas production and associated discharges (Kendall 2012).

Incineration

Incineration is thought to be the second-most popular end-of-life option after landfilling, with 800 metric tons of waste having been burned since polymers first entered the market (<https://assets.kpmg/content/dam/kpmg/uk/pdf/2019/06/to-ban-or-not-to-ban-v6.pdf>). Generally speaking, incineration (also known as burning) describes a reaction of waste at high temperatures and in the presence oxygen. It is a catalytic, exothermic reaction, and the heat radiation and free radicals it produces are what keep it moving forward. The irreversible chemical breakdown known as pyrolysis, on the other hand, is caused by an enhancement in temperature in the absence of oxygen and without the use of any oxidation processes. Municipal solid waste incineration is a technique for handling leftover that decreases the volume and quantity of solid waste that requires to be landfilled while utilizing the material's energy content. In Europe, it is a common technology (Oppelt 1987; Lam et al. 2010; Sabbas et al. 2003). In the Netherlands, accumulated polymers which cannot be recovered or reused are burned because landfilling is not allowed (Somers et al. 2021).

The researchers choose burning as a way to dispose, particularly for natural polymers, not only because of its high recycling efficiency but also because the thermodynamic efficiency yields the most return with the least effort and money. Since biopolymer includes a lot of bio-based components, incineration produces more CO₂-neutral energy. Additional energy recovery is appropriate irrespective of the input materials' foundation, renewability, or recyclability of all forms of bioplastics and their composites (Endres and Siebert-Raths 2011). Owing to the renewable calorific value, PLA biopolymers have always been the best alternative with a reduced carbon footprint when it comes to the end-of-life method which is most frequently utilized in Europe (incineration). After usage, the carbon footprint for PLA bio-composite items with low humidity may even be negative. With more energy needed for incineration due to a high moisture content, there are more CO₂ emissions. Biodegradable bioplastics have a smaller carbon footprint and can divert organic material for these purposes (PLA and PLA 2020).

Littering

Littering means throwing away of products carelessly. This ambient disposal is mostly observed in parks and other places. The usage of appropriate biodegradable polymers would ensure the products' eventual dissolution and degradation; in this

way, littering is done without taking into consideration the degradability and environmental impacts. If biopolymers were employed, the throw-away mentality would expand, and consumers would cease to distinguish between plastic garbage that degrades and waste that doesn't degrade without considering the degradability and environmental impacts (Endres and Siebert-Raths 2011).

Future Prospective

Researchers investigate anticipated future developments in the production and recycling of polymers and their potential effects on the accomplishment of recyclability objectives. The characteristics and behavior of the compound should be examined in order to determine the alternatives and constraints for the improvement of the recyclability of biopolymer products in the future. To manage bio-based waste effectively in the future, it is important to understand how these products are currently managed. Waste made of biopolymers must not be dumped in landfills, even if they are biodegradable. There are fundamental requirements and issues when handling bio-based polymers. To ensure the quality required when employing recycled materials obtained from biopolymer waste, the major industries must harmonize quality requirements at the level of the circular economy. The extensive use of recycled biopolymer manufacturing is thus supported by these standards, which may also contribute to a rise in the quantity of novel bio-based materials whose recycling will be possible by a number of cascade usage phases. The assessment approach thus emphasizes the identification of pertinent polymer advances and ranges for standardization (Hildebrandt et al. 2017). Disposal of the waste material should be the last option because when a material deteriorates, their products can be harmful for the environment. The management of individual waste streams following waste segregation is a key necessity, as a mixture of all home garbage and industrial waste is invalid for processing to recover materials. The used products that a consumer wants to discard must be tagged appropriately in order to make the waste management easy. This will make it possible to identify recycling routes, which will eventually boost the throughput of secondary raw resources. The selection of suitable method for the waste management will create multipurpose avenue for improved reuse, recycle, and disposal roadmap to manage solid waste in a cost-effective way.

Conclusion

Modern waste management must deal with a brand-new kind of garbage called biopolymers. There are numerous strategies to convert biopolymer waste treatment through recycling, salvaging, and reuse to decrease waste and boost revenue. An increasing drive toward a sustainable future includes hazardous waste sustainable or "green" management techniques that endeavor to preserve nature, reduce overall resource use, and improve energy efficiency. It has been demonstrated that

biomaterial sector can gain substantially from the implementation of waste management strategies that focus on appropriate dumping of waste and their reuse and recycling. To manage bio-based waste effectively in the future, it is important to understand how these products are currently managed. Industrial biodegradable polymers like PLA presently have minimal, dispersed waste streams, and their existing usage rates do not justify the establishment of an independent PLA recycling stream. As their large-scale commercial application takes off, the handling of biopolymers in the waste stream will become a critical concern.

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Safety Issues, Environmental Impacts, and Health Effects of Biopolymers

52

Ragitha V. M. and Lekshmi K. Edison

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Abstract

Petroleum-based polymers are linked to a variety of health and environmental issues throughout their life cycle, such as pollution, greenhouse gas emissions, persistence in marine and terrestrial habitats, etc. On the other hand, biopolymers are a fast-expanding class of polymeric materials that are frequently offered as substitutes for traditional plastics made from petroleum. However, there is a need to assess the true health and environmental impact of using biopolymers because they have also been connected to significant health and environmental problems like greenhouse gas emissions and adverse land use change. Though numerous evaluations include biopolymers, very few fully and concurrently analyze the advantages and disadvantages of using biopolymers for the environment. The

R. V. M.
KSCSTE-Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Thiruvananthapuram,
India

L. K. Edison (✉)
Department of Comparative, Diagnostic and Population Medicine, College of Veterinary Medicine,
University of Florida, Gainesville, FL, USA
e-mail: edison.le@ufl.edu

current chapter deals with the safety aspects of biopolymers in human health and the environment.

Keywords

Polymers · Biopolymers · Health impact · Environmental impact

Introduction

Polymers are macromolecules obtained through the association of monomers linked together with chemical bonds (Elias 1997). The word polymer originates from Greek and means many (poly) parts (meres). A polymer molecule size usually varies between 10,000 and 1,000,000 g/mol (St. Pierre 1972; Elias 1997). Polymers have immense applications in our day-to-day life, and even a new branch raised as polymer chemistry and people from different fields such as biomedicine and pharmacy, textile technologists, mechanical engineers, biochemists, and biophysicists contribute significant information that make polymer as an unavoidable thing in different fields including medicine (Pillai and Panchagnula 2001).

Polymers can be classified into two based on their origin: synthetic polymers and biopolymers (Fig. 1). Synthetic polymers are man-made, and biopolymers are polymeric macromolecules found in living organisms.

Synthetic polymers are structurally similar to biopolymers but are chemically synthesized for special uses. The physical forces included in the formation of synthetic polymers are similar to that of biopolymers such as dipole-dipole interactions, hydrogen bonds, and London forces (Ouellette 2015). Hydrogen bonding between amides and polyamides is responsible for the strength of synthetic polymers. Different polymerization methods like addition, copolymerization, and condensation are used, and products showed different characteristics based on their polymerization methods (Ouellette 2015). The synthetic polymer industry showed

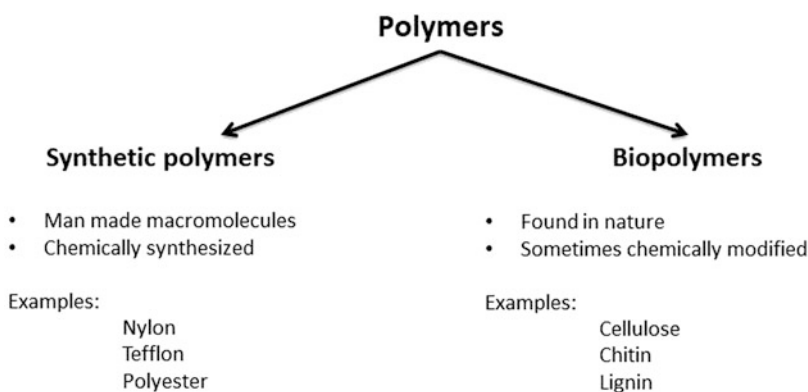


Fig. 1 Classification of polymers

Table 1 Comparison between biopolymers and synthetic polymers (Shrivastava 2018)

Biopolymers	Synthetic polymers
Origin from nature	Man-made synthesis
Similar repeating units	Identical repeating units
Formation by biological processes	Formation by chemical process
Contains carbon, hydrogen, and nitrogen	Contains mostly carbon
Biodegradable	Only few are biodegradable
Sensitive to heat, chemicals, etc	Resistant to heat, chemicals, etc
Environmental friendly	Produce environmental issues

exponential growth because of its advantages over biopolymers. Table 1 illustrates the comparison between biopolymers and synthetic polymers (Shrivastava 2018).

Basic raw ingredients, termed monomers, are used to create synthetic polymers, which are collectively referred to as plastics (polycondensation, polymerization, and polyaddition) under specific circumstances. Plastics can take on a variety of shapes, including pipes, thin sheets, bottles, and bags, among others. They also resist corrosion and other chemical variables, are easy to handle, and work as thermal and electrical insulators; therefore, they have spread and are utilized in practically all spheres of human endeavor. Despite its undeniable usefulness in daily living, synthetic polymers turn into garbage after being utilized. 25 million tonnes of plastic are thought to be added to the environment each year, which can stay the same for a time period of between 100 and, due to its relatively slow disintegration, 500 years consisting its breaking up into smaller pieces. These particles cause issues with water, air, and pollution of the soil that directly affect the health and the environment. Moreover, the highest portion of plastics is made using fossil fuels, which places an undue burden on nonrenewable resources.

From Table 1, we could easily identify that synthetic polymers cause environmental problems, which prompted people to consider an alternative solution to synthetic polymers that has a lower environmental impact. Thus, people are again concentrated on biopolymers and think about the procedures that can be adopted to modify and make natural polymers with the advantages of synthetic polymers. Due to the aforementioned, the so-called biopolymers have been created, which have thermoplastic and physicochemical qualities comparable to petroleum-based polymers with the exception that they are biodegradable. The use of these resources raises a number of issues and problems, though, as the use of renewable raw materials does not necessarily indicate sustainability. We have to first understand the reason of emergence of biopolymer as an option. For this we have to understand the advantages and disadvantages of biopolymers and synthetic polymers, and that are listed in Table 2 (Muhamad II et al. 2014; Satturwar et al. 2003; Kulkarni Vishakha et al. 2012; Gavasane and Pawar 2014; Muxika et al. 2017; Muslim et al. 2013; Ahsan et al. 2018; Jesus et al. 2018; Maghsoudi et al. 2020; Jani et al. 2009).

The emerging trend of identifying and modifying biopolymers increases as it is believed that biopolymers are devoid of health and environmental effect of synthetic

Table 2 Advantages and disadvantages of biopolymers and synthetic polymers

Polymer	Advantages	Disadvantages
Natural polymers	Water-soluble Abundance Less toxic Biocompatible Biodegradable Less side effects Exerts less toxicity along with drugs Chemical modification possible Low cost Low angiogenesis Low immunogenesis Mucoadhesiveness	Chemical molecules are vary based on climate, geographical conditions, availability of nutrients, and thus chemical constituents different from batch to batch Microbial contamination is high Adulteration needed Heavy metal contamination Time consumption for extraction and purification Low mechanical strength Sensitive to heat and chemicals Recycling and reuse not possible
Synthetic polymers	Mechanical stability Durability Inertness Tunable Beneficial tailoring properties Reproducibility Bioabsorbable Environmental stability Biocompatible	Intrinsic biocompatibility and bioactivity are absent Toxic Oncogenesis Inflammation and immune response High production cost Water-insoluble

polymers. This chapter tries to understand the classification of biopolymers, their applications, and the impacts of biopolymers on health and the environment.

Biopolymer

Biopolymers are organic substances found in living organisms, and the term originates from Greek words bio and polymer (Ezeoha 2013). Biopolymers are biocompatible macromolecules that have immense applications in our day-to-day life. Biomolecules include nucleic acids, lipids, carbohydrates, proteins, etc. Polymers such as plastics are synthetic polymers but have more lifelong benefits compared to biopolymers.

Biopolymers can be classified based on different parameters, and one of the most important features for classification is based on their monomer, their origin, and source (Fig. 2). Based on their monomeric units, biopolymers come under three groups: polynucleotides, polypeptides, and polysaccharides (Pakawadee 2012). Based on origin, biopolymers can be classified into polyesters, proteins, polysaccharides, lipids, surfactants, polyphenols, and specialty polymers from natural fats and oils (Kaplan et al. 1994). Based on source, polymers can be classified into two and named as natural biopolymers and synthetic biopolymers (Baranwal et al. 2022).

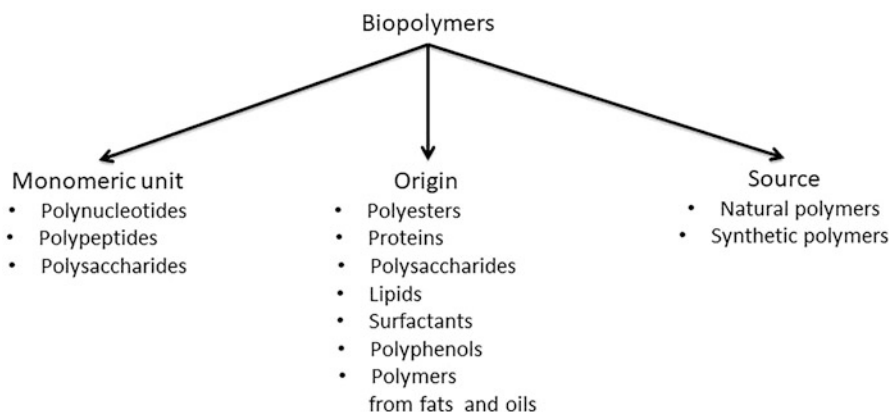


Fig. 2 Classification of polymers based on origin, source, and its individual unit

Based on the source of polymers, biopolymers are classified into natural biopolymers. Natural polymers are obtained from living organisms and are good replacements for synthetic petroleum-based plastics. They are very much accepted by the people because of their abundance, renewability, cost-effectiveness, biocompatibility, and biodegradability. Starch, cellulose, chitosan, gelatin, carrageenan, and alginates are good examples of natural biopolymers. They are isolated from bacteria, fungi, plants, and animals.

Even though the abovementioned features of natural biopolymers are attractive, there are certain disadvantages that make us think about other nature-friendly materials. The disadvantages include low tensile strength and barrier properties (Baranwal et al. 2022). Since the origin is biological, some also exert immunogenicity and angiogenic properties (Shankar and Rhim 2018; Dmour and Taha 2018). And a multistep purification process is also required after their isolation from the source. Thus, modification of natural polymers is a thought that came to exist, and people tried to modify natural biopolymers to overcome the side effects associated with them. And this leads to the development of a new category called synthetic biopolymers. Polylactic acid, polycaprolactone, and polyvinyl alcohol are examples of some synthetic polymers (Kaplan et al. 1994).

Synthetic biopolymers can be defined as polymers that are produced from synthetically produced monomers or ones that are natural polymers modified with chemical processes (Jenkins et al. 1996). Biodegradability is the added advantage of synthetic biopolymers over synthetic polymers produced from nonrenewable sources like petroleum. Thus, synthetic biopolymers got much attention over both synthetic and natural biopolymers. Synthetic biopolymers are even better than natural biopolymers because of their higher stability and durability for their transportation and use. The most important advantage is that they are sterilizable and heat and chemical resistant, and so they can be used in the medical field.

Applications of Biopolymers

Natural and synthetic biopolymers have significant roles in our day-to-day life. Each and every field of our life is now connected to biopolymers, and some fields are listed below (Fig. 3).

Automotive industry: Biopolymers are nowadays used in automotive industry. Plastics are vital to the automotive industry because they are a readily available and manageable material, yet consumers are increasingly seeing pictures of mounds of trash and enormous floating islands of plastic. The issues with ordinary plastic might be partially resolved by the use of bioplastics. Polylactic acid, a polyester developed from cellulose, is proven as a replacement for synthetic polymers (Barillari and Chini 2020). By fermenting sugar, a raw material that is widely accessible in large quantities practically in any place, lactic acid for biopolymers is produced. Corn (USA), starch (Asia), and sugarcane all play important roles depending on the region. A great degree of efficiency can also be achieved in the conversion of sugar to the ultimate product: only about 1.6 kg of fermentable sugar is needed as a raw material to produce 1 kg of PLA (Lovett et al. 2017). This indicates that PLA are biopolymers that can be created using a technology that is very energy- and resource-efficient. PLA are with low softening skill, chemical resistance, aging resistance, and workability. These features prevent them from being a good alternative in automotive industry (Koltzenburg et al. 2014; Maier and Schiller 2016; Notta-Cuvier et al. 2014). Modification of conventional PLA with different new chemical treatment options makes it as a suitable material for automotive industry.

Textile industry: Textile can be defined as the network of intangible fibers (Law 2004). Natural, semisynthetic, and petroleum-based synthetic fibers are used for the production of textile fabrics (Karthik and Rathinamoorthy 2017). Problems

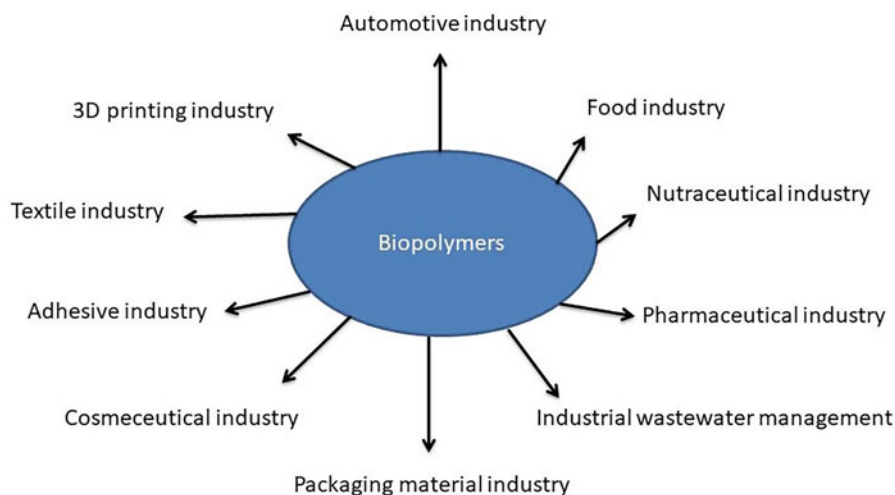


Fig. 3 Application of biopolymers

associated with conventional and synthetic fibers lead to the use of biopolymers. Biopolymers are used in textile industry as binding agents; levelling agents, providing uniform coloring to the cloth material; viscosity modifier; thickening agent; and textile finishing and for coloring.

Adhesive industry: Adhesive itself has huge applications in other industries like medical field. Renewable materials are now used as a source of adhesive synthesis (Gesthuizen and Robert 2018). Vegetable oils are one of such biopolymers with increased adhesive property because of its hydrophobicity. Renewable building blocks such as sebacic acid, itaconic acid, succinic acid, and even glycerol and fatty acids from vegetable oils are used as either biopolymers or building molecules (Heinrich 2019). Resin is another biopolymer which is used in pressure-sensitive adhesives and wooden floorboard adhesives (Quack and Yaacoub 1998).

3D printing industry: The rapid development of products with complex architectures is made possible by the groundbreaking additive manufacturing process known as three-dimensional (3D) printing. This review discusses the current state-of-the-art biopolymers (“protein- and carbohydrate-based materials”) used in pharmaceutical, bioengineering, and food printing, as well as the primary biomacromolecular structure reinforcement techniques used in the creation of 3D structures. There are also some viewpoints and key restrictions on the use of biomaterials in sophisticated 3D printing processes (Shahbazi and Jäger 2020). Biopolymers are the most often used materials for 3D printing applications due to the enhanced mechanical strength of the printed architectures and improved ink flow behavior. By utilizing the ability to change ink viscosity, bio-based polymers could enhance the resolution of deposited layers and printing accuracy and subsequently generate well-defined geometries. Protein-based biomaterials like gelatin, collagen, zein, soy, casein, and wheat flour are used in blend with other substances like starch, potato granules, etc. Chitosan, starch, pectin, and cyclodextrin are some of the polysaccharide-based biopolymers used for 3D printing (Shahbazi and Jäger 2020).

Packaging material industry: Flexible packaging typically uses biodegradable bioplastics, whereas rigid packaging typically uses bioplastics that are not biodegradable. The market for biodegradable, semi-durable, and durable bioplastics utilized in consumer and industrial applications is the main emphasis of the future of bioplastics (European bioplasticsb 2017). In place of traditional polymers, biodegradable polymers can be utilized for modified atmospheric storage (MAP) of fruits and vegetables. Starch-based polymers have wide acceptance as a packaging material for food, and some such polymers are polylactic acid, polyhydroxyalkanoates, etc. (Weber et al. 2002).

Biopolymers and health industry: As we mentioned above, biopolymers are used in different fields of health like pharmaceutical field and medicinal field. New technology always aims for maintaining health and life expectancy of humans. Synthetic and semisynthetic polymers are replaced by biopolymers, and they are now used in almost every field of medicine and treatment options. Drug delivery systems and surgery related processes like cell proliferation, isolation, covering, occlusion, adhesion, tissue guiding, etc. all hugely depend on biopolymers (Neuendorf et al. 2008; Chen et al. 2018; Tsai et al. 2018; Park et al. 2017). New

processes and techniques have been introduced to make biopolymers as the most suitable material for biomedical applications (Yahya et al. 2021). The applications of biopolymers in the medical field are soft tissues replacement, artificial hearts, bone cement, pacemaker, breast implants, extracorporeal oxygenators, contact lenses, sutures, adhesives, coating of pharmaceutical medications, blood substitutes, encapsulations, joint replacement, dentistry, drug delivery, cardiac assist devices, targeting sites of tumors and inflammations, tissue engineering, etc. (Pattanashetti et al. 2017; Reddy et al. 2015; Wróblewska-Krepsztul et al. 2019; Gagliardi et al. 2021). Biopolymers that are used in different fields and their application in the medical field are listed in Table 3.

Health and Environmental Impacts of Biopolymers

The impacts of biopolymers on human health and the environment depend on the type of feedstock used for biopolymer production. The feedstocks are divided into three generations: first, second, and third (Ahorsu et al. 2018).

First generation: carbohydrate-rich feedstocks include corn, wheat, sugarcane, sugar beet, potato, plant oil, and rice and are used as human and animal food.

Second generation: feedstocks include nonfood crops like cellulose or byproducts of the first-generation feedstocks like corn stover or sugarcane bagasse.

Third generation: biomass derived from industrial or municipal water, algal biomass, and food industry byproducts.

The first-generation feedstocks do not produce much health effects at lower concentrations since they are consumed as food products. But they have a high impact on the environment; for the production of feedstocks, huge agriculture land is required which affects the ecological balance (Tenenbaum 2008). Like the first-generation feedstocks, the second-generation feedstocks do not produce much conflict on health unless it is a residue of the first-generation feedstocks. A high cost is required for the processing of the second-generation feedstocks (Rosenboom et al. 2022). Both generations use fertilizers, herbicides, and pesticides which in turn causes serious health issues in humans and animals. The third-generation feedstocks do not need fertilizers and pesticides. The third-generation feedstock does not need fertilizers and pesticides but industrial and municipal wastes, phytoplankton and algae contaminated with toxic substances like heavy metals affect health of living organisms. The process of removing such toxic substances is nearly impossible, and the cost and time of production are more (Atiwesh et al. 2021).

Overall, life cycle evaluations have shown that the initial generations of bio-based plastics have lesser substantial negative impact on human health and the environment than their petrochemical competitors (Brizga et al. 2020). Not all categories, nevertheless, fall within this category. When compared to petrochemical materials, the feedstock growing phase has a higher need for water and arable land (Haider et al. 2019; Bishop et al. 2021; Atiwesh et al. 2021). There are linked environmental effects when land use changes from a natural environment to biomass agriculture. Because native plants and animals are displaced or have less space to establish a

Table 3 Biopolymers and its applications in health

Biopolymers	Applications	Reference
Collagen	Suture, hemostatic agent, bone graft substitute, drug delivery	Burke et al. 1981 ; Royce et al. 1995 ; Merodio et al. 2002
Natural poly(amino acids)	Drug delivery vehicle, tissue engineering scaffolds, thermosensitive polymers	Akio et al. 2008
Elastin	Injectable drug delivery system	Chilkoti et al. 2006
Fibrin	Treatment of chronic wounds	Wang et al. 2001 ; Grassl and Tranquillo 2006 ; Terasaka et al. 2006
Hyaluronic acid	Wound dressing, synovial fluid substitute, bone graft for bone fracture	Prestwich et al. 1998 ; Mori et al. 2004
Chondroitin sulfate	Antiinflammatory properties, wound dressing, production of hydrogels for biomedical applications	Iovu et al. 2008 ;
Chitin and chitosan	Wound healing accelerator, chitosan conjugates for cancer treatment, scaffolding material, antibacterial and hemostatic properties	Onishi et al. 2001 ; Illum et al. 2001 ; Jayakumar et al. 2007 ; Hein et al. 2001 ; Dodane and Vilivalam 1998
Alginic acid	Wound dressing, drug delivery, protein delivery system, wound healing	Salgado et al. 2002
Xanthan	Controlled drug delivery	Shingel and Marchessault 2006
Gum arabic	Suspending and emulsifying agent, reduces blood cholesterol, blood pressure	Seema and Arun 2015
Starch	Controlled drug delivery, tablet disintegration	Matveev et al. 2001 ; Lu et al. 2009
Cellulose	Controlled pharmaceutical delivery systems, hydrogel production	Swabrick and Boyan 1991
Pectin	Suspending and thickening agent, reduce blood cholesterol, treat gastrointestinal diseases, tissue engineering applications	Munarin et al. 2011 ; Linshu et al. 2004
Carrageenan	In drug solution for increasing the permeability of drugs	Pacheco-Quito et al. 2020
Poly(α -esters)		Burg 2014
Polyglycolide	Sutures, as bone internal fixation devices	Singhvi et al. 2019 ; Gorrasi and Pantani 2018
Poly lactides	Drug delivery, scaffolding material for tissue regeneration, suture development	Fu et al. 2000
Poly(lactide-co-glycolide)	Multifilament suture	Makadia and Siegel 2011 ;

(continued)

Table 3 (continued)

Biopolymers	Applications	Reference
Polycaprolactone	Contraceptive device, monofilament suture	Thomas 2011 ; Malikmammadov et al. 2018
Polydioxanone	Treating small bone and osteochondral fragments	Martins et al. 2020 ; Chen et al. 2006
Poly (3-hydroxyalkanoates)	Drug delivery vehicle, surgical implant	Hu et al. 2003 ;
Poly(ester amide)	Site-specific delivery of small hydrophobic drugs and peptides	Dias et al. 2006 Hu et al. 2003 ; Khanna and Srivastava 2005 ; Jeon et al. 2007 Francis et al. 2010
Poly(orthoesters)	Drug delivery especially for ocular applications	Mansour et al. 2010
Polyanhydrides	Localized drug delivery vehicle in chemotherapy for brain cancer and osteomyelitis	Basu and Domb 2018 ; Gunatillake and Adhikari 2003
Polypropylene fumarate	Bone tissue engineering applications	Armmrtno et al. 2010 ; Heller and Gurny 1999
Poly(alkyl cyanoacrylates)	Tissue adhesive for skin application	Andrianov and Payne 1998
Polyphosphazenes	Scaffold for tissues engineering	Langer 1997
Polyphosphoester	Drug delivery applications, scaffold for tissue engineering	Naser et al. 2021

home, biodiversity declines as natural areas are converted to agriculture (Haider et al. [2019](#); Atiweish et al. [2021](#); Nanda et al. [2022](#)). The usage of pesticides and fertilizers throughout the growth stage of the feedstock is frequently observed as the primary source of concern for human health and the environment. Pesticide exposure can occur orally, topically, or through soil, water, or air contamination. Harmful algal blooms, which produce toxins and other toxic substances in water, are associated to pesticide exposure (Nanda et al. [2022](#)).

Respiratory issues, neurological impacts, stomach or liver disorders, and skin irritation are some of the negative health effects of exposure. Additionally, 99 percent of utilized pesticides and 95 percent of applied micronutrients for agrochemicals bounce off the plant and are wasted (Duarte et al. [2016](#)). Due to the creation and application of fertilizers, the eutrophication and acidification consequences of the first-generation feedstocks are often harsher than those of their fossil fuel counterparts. Eutrophication can result from fertilizer nutrients entering the bodies of water, and biopolymers' acidification action is aided by emissions from fertilizers into the soil (Nanda et al. [2022](#)). In particular, even when accounting for the CO₂ emission at the end of the product's life and the net CO₂ effect from plant growth, the use of nitrogen fertilizers increases the global warming impact (Nanda et al. [2022](#)).

The food vs. fuel dispute is significant since the first-generation feedstocks are also utilized as food, which results in biopolymer synthesis being in direct conflict with food production (Duarte et al. [2016](#)). An adverse economic effect might be

higher food costs, which would increase food insecurity, resulting from increased demand for corn and sugar for the development of bioplastics. One of the most important effects of the food vs. fuel debate on public health is identified as being food security (Duarte et al. 2016). Currently, there is little competition between industrial production and food and feed production for arable land. Less than 0.02% of the world's agricultural land is now dedicated to the production of bioplastic feedstock (Duarte et al. 2016). The agricultural sector is anticipated to remain at a low 0.06% in 2025 despite the forecasted increased growth in the bioplastic market (Duarte et al. 2016). For background, around 91% of the agricultural acreage is used for grazing, food, and feed (Duarte et al. 2016). Even though there is no competition between the productions of food, feed, and biopolymer feedstock, there are still rising food demands and ongoing strains on the food supply. The resources available for industrial production will be affected.

Health Impacts of Biopolymers

The health impacts of biopolymers are still under study, and a long duration is necessary to understand the toxic effects of biopolymers. The three primary reasons why biopolymers are harmful are well explained in the literature: The first is that they cause an excessive amount of granulomas, an inflammatory response, in the body due to the immune system's recognition of the biopolymer as a foreign substance item, and encapsulating it causes a defensive response it. Second, they can move away from where they were applied, resulting in issues on other distant places. The third reason is because they frequently fail to meet the minimal standards for health controls, which raises the danger. Local reactions include fibrosis and lesions, and modifications in skin texture are key additional effects of biopolymers. When this occurs, it can occasionally result in death from an embolism when chemicals are directly injected into the blood vessel (Samir et al. 2005a; Peng et al. 2011).

Certain studies reported the deleterious effect of biopolymers on health. Nanocellulose is a biodegradable biopolymer derived from bacteria and algae and has been used for their pharmaceutical and cosmetic effects (Terech et al. 1999; Siqueira et al. 2009; Lima and Borsali 2004; Grunert and Winter 2002; Samir et al. 2005b; Adamis 1997; Cullen 2002). Several studies reported the pulmonary toxicity of nanocellulose (Kovacs 2010; Tatrai 1995; Warheit et al. 1998, Warheit et al. 1998, Roman et al. 2009; Yanamala et al. 2014). In human endothelial cells, it is nontoxic at a concentration of 0–50 µg/mL, and above that it exerts physiological changes (Eroglu et al. 2017). Other studies also supported the abovementioned findings, and they proved that nanocellulose induces fibrous bronchiolitis, pulmonary granulomas, inflammation, alveolitis, etc. (Radu et al. 2019).

Calcium hydroxyapatite causes infection, arterial embolization, swelling, nodule, blindness, necrosis, and blanching as clinical complications (Taghizadehghalehjoughi Taghizadehghalehjoughi et al. 2018; Voss et al. 2020; Velard et al. 2015; Bharadwaz and Jayasuriya 2020). In in vitro studies, exposure of calcium

hydroxyapatite increases inflammatory markers like TNF- α , IL-8, IL-10, and IL-1 in monocyte culture (Morhenn et al. 2002).

Collagen showed discoloration, skin necrosis, granuloma formation, blindness, and foreign body reactions as clinical manifestations (Velard et al. 2015; Farina et al. 2018). In *in vitro* studies, phagocytosis of collagen by immune cells released TNF- α (Saththianathan et al. 2017).

Hyaluronic acid clinically showed swelling, pain, arterial embolization, bleeding, blanching, Tyndall effect, infection, and nodule formation (Taghizadehghalehjoughi et al. 2018; Voss et al. 2020; Velard et al. 2015; Bharadwaz and Jayasuriya 2020; Tesar et al. 2006). Hyaluronic acid helps bacterial growth in biofilm and contaminated tissue engineering material (Jeong et al. 2021). In murine cell culture, it acts as innate immune agonist (Jeong et al. 2021; Centeno and Young 2006; Saththianathan et al. 2017).

Poly-L-lactic acid causes infection, swelling, blindness, bleeding, granuloma, nodules, etc. (Taghizadehghalehjoughi et al. 2018; Voss et al. 2020; Velard et al. 2015; Cabral et al. 2020). *In vitro* studies showed that cells treated with poly-L-lactic acid cause a reduction in cell proliferation and viability (Shoenfeld and Agmon-Levin 2011; Ameratunga et al. 2017).

Autoimmune/inflammatory syndrome induced by adjuvants (ASIA) is another health condition induced by adjuvants that are used along with biopolymers (Colafrancesco et al. 2014). Silicosis, postvaccination phenomena, macrographic myofasciitis syndrome, Gulf War syndrome, sick building syndrome, Sjogren's syndrome, and autoimmune thyroiditis have been reported as part of ASIA (Wadat et al. 2016; Vera-Lastra et al. 2013; Hawkes et al. 2015). These conditions lead to clinical characteristics such as chronic pain, fibromyalgia, chronic fatigue syndrome, cognitive dysfunction, myalgia, fever, arthralgia, and autoantibody positivity (Pachón et al. 2021; Tchounwou et al. 2012; Jamshaid et al. 2017).

Biopolymers are preferable to synthetic polymers in terms of their safety, but they also possess health issues, such as bioaccumulation, carcinogenetic properties, air pollution, and respiratory illness. Technologies have to be developed further to remove the health impacts of biopolymers.

Environmental Impacts of Biopolymers

Invention of biopolymers helps to avoid many environmental issues including the removal of heavy metals and dye, cleaning of oil spillage, carbon dioxide adsorption, and wastewater treatment, etc.

Removal of heavy metals: Heavy metals are needed for various industrial processes for energy production (Anastopoulos et al. 2017). Thus, large quantities of industrial waste containing heavy metals are generated. And these wastes are sometimes not even processed further and dumped into the environment which leads to the contamination of water, air, soil, and biosphere. They are toxic and nonbiodegradable, and one of the most serious issues associated with heavy metals is their persistence and bioaccumulation. They enter the food chain and reach the

human body through contaminated plants and animals. Heavy metal contamination is a serial health issue that causes birth defects, skin irritations, liver toxicity, stomach cramps, nerve system damage, and autoimmune diseases. Industrial wastes are subjected for heavy metal removing using processes like coagulation, biological treatments, chemical precipitation, membrane filtration, adsorption, etc. Adsorption is the most effective method, and biopolymers are nowadays used as a cheap and energy-efficient method, and cellulose, chitosan, and chitin are used as heavy metal adsorbents (Zhang et al. 2016; d'Halluin et al. 2017; Chequer et al. 2013). Biopolymers in combination with other compounds are proven to be effective in removing heavy metals. For example, cellulose with ethylenediaminetetraacetic acid removes heavy metals from water and is effective in removing lead, cadmium, nickel, zinc, etc. (Yagub et al. 2014).

Removal of dye: Dye is an important component in textile, paper, leather, printing, and paint industry, and about 10–20% of totally produced dye is discharged directly into the water sources (Zhou et al. 2011; Li et al. 2013; Zhu et al. 2007). Among the dyes, mostly colored and acid dyes are very dangerous because it cannot be easily removed from the water bodies especially using conventional methods. Organic dye discharge into water bodies has caused immediate and ongoing issues for human health and ecosystems. For instance, the release of those organic dyes might result in eutrophication, non-esthetic pollution, imbalance in the aquatic biological systems, chronic toxicity, carcinogenicity, and neurotoxicity toward humans and animals, among other negative effects. Chemical precipitation and adsorption (Patel and Vashi 2012; Teh et al. 2016), coagulation and flocculation (Chan et al. 2009; Işık and Sponza 2006), aerobic and anaerobic treatment (Alaoui et al. 2015; Ciardelli et al. 2001), electrochemical oxidation and reduction (Zhang et al. 2011), membrane separation (Dariani et al. 2016), H_2O_2 /ultraviolet (UV), photocatalysis (Rosario-Ortiz et al. 2010; Huang et al. 2014; Raghu and Basha 2007), ultrafiltration (Zhang et al. 2011), ion exchange (Zhuang et al. 2010), sonochemical degradation (Lim et al. 2006), Fenton and heterogeneous Fenton-like catalysis (Hsueh et al. 2005; Matheswaran and Raju 2010; Serpil 2019), and electrolysis (Liu et al. 2015) are some of the commonly used technologies for removing organic dyes from wastewater (Ruan et al. 2018).

Biopolymers are used for removing dyes because of their sorbent properties. Aerogels are used to remove cationic dye malachite green from water sources, and cellulose-based bioadsorbent was used to remove anionic dye (acid blue) and cationic dye (methylene blue) along with acrylic acid and acrylamide (Doshi et al. 2018). 2,3-Dialdehyde cellulose-chitosan showed 100% of Congo red dye removal (Tu et al. 2018). More technologies are being developed to identify the best adsorbent for different categories of dyes (Ruan et al. 2018).

Oil spilling and solvent spilling cleanup: Oil or solvent spilling is the discharge of oil or solvent accidentally or intentionally to the environment and which causes intense water pollution (Ruan et al. 2018). The production of oils and solvents results in huge costs and increased usage demanding for its transport into different parts of the world. One of the most important things in oil spillage other than money loss is its impact on water bodies. To protect the aquatic environment and subsequent

contamination to human body, the spillage should be removed as soon as possible. Different approaches are used like bioremediation, physical diffusion, mechanical recovery, and in situ burning (Xu et al. 2018; Cheng et al. 2017). Different studies proved the effectiveness of biopolymers to adsorb oil/solvent filling, and one such study showed that magnetic hydrophobic polyvinyl alcohol-cellulose nanofiber aerogels absorb oil effectively (Le Quéré et al. 2012). Cotton/cellulose aerogels also remove oil and organic solvents (Cassie and Baxter 1944).

Carbon dioxide adsorption: Synthetic polymers and huge carbon monoxide and carbon dioxide emission lead to global warming and climate change (Wang et al. 2011). By 2030, the carbon dioxide levels will be in the range of 600–1550 ppm (Leung et al. 2014). CO₂ is a greenhouse gas that has inherent ability to increase temperature which leads to the rising of sea levels, changing rainfall patterns, and weather changes including flood, freezing winters, and severe heat waves and droughts (Ruan et al. 2018). Carbon dioxide capture, storage, and utilization technique is widely used to control CO₂ emissions (Dassanayake et al. 2016; Sehaqui et al. 2015). Different materials are used for this purpose, and biopolymer also showed good results for the absorption of CO₂. Activated carbon monoliths from cellulose-based gel have CO₂ absorption properties at low temperature (Rahman and Hasan 2019). Nanofibrillated cellulose-polyethylene foams are reported to have direct absorption of CO₂ (Kehinde et al. 2020).

Effect of Biopolymers on Our Environment

The development of new solutions to ease our human life mostly ends up with issues in the environment. That is why sustainable development has its huge acceptance. Invention of artificial or synthetic polymers is thought to be a major advantage for us which overcomes the disadvantages of natural polymers which include the variability associated with the source, complex structures, tedious and costly extraction process, etc. Synthetic polymers that are mostly developed from nonrenewable petroleum sources have good strength, resistivity, and flexibility, and its most favorable feature is its chemical and biological inertness. Chemical and biological inertness is responsible for its long-lasting usage and is one of the most favorable reasons for its wide acceptance. Even though it was considered as the most beneficial advantage of synthetic polymers, within a short period of time, the blessings turn into a curse; because of their inertness to chemical and biological reactions, the synthetic polymers became a problem as a waste material and cause detrimental effects on the environment. Every year, the demand for synthetic polymers is also increasing. This leads to thinking about modified biopolymers that are compatible with synthetic nonbiodegradable polymers except for its biological degradation. The advantages of synthetic biodegradable polymers can be understood by knowing the detrimental effects of nonbiodegradable polymers on the environment. Synthetic nondegradable polymers are artificial polymers like plastics that are developed from fossil fuels and synthetic biopolymers that are modified from natural polymers or produced from chemically synthesized synthetic monomers (Blettler et al. 2018).

The nondegradable polymers from nonrenewable resources have deleterious effects on the environment. The accumulation of nondegradable polymers contaminates water, land, and air which further affects the lives of organisms and the ecosystem (Kaza et al. 2018).

The aquatic system is polluted with the accumulation of polymers like plastics in water resources (Charles et al. 2008). Humans either directly introduce synthetic, nondegradable polymers into water sources or indirectly through the soil. When plastics are released into the ocean as sediments, a concentration that is far more hazardous than that found in sea water is created automatically. Heavy metals and organic chemicals created by hazardous pollution brought on by this plastic debris being dumped in the water are priority pollutant metals. By ingesting this contaminated seafood, humans can become exposed to this pollution through their tissue and skin as well as through aquatic life. Because they spend most of their time near the ocean on land or on the water surface, drinking sea water, or eating these infected seafood, ocean birds are also at risk (Charles et al. 2008).

According to the US National Institutes of Health, 44 percent of seabird species are known to have consumed synthetic polymers that were mistaken for food (Saleh and Gupta 2016). Millions of seabirds per year perish as a result of this ingestion. This is one of the most frequent environmental issues connected to the pollution of synthetic polymers. Given that shorebirds are crucial for maintaining fish and crustacean population numbers, the widespread loss of shorebirds poses a serious environmental issue.

Persistent organic pollutants, such as pesticides, DDT, and toxaphene, are well-known poisons that linger in the environment for many years. In a 2007 investigation, scientists from the University of the Pacific took samples of synthetic polymers from coastal locations in the northern Pacific Ocean and discovered hazardous chemicals in each sample. These artificial polymers pose a threat to the sustainability of human food-producing ocean fisheries by continuously secreting toxic compounds into fish and other species.

Synthetic polymers can cause environmental issues throughout the production process in addition to their obvious ocean contamination. According to the Environmental Working Group, the DuPont chemical plant has been leaking contaminants used to make Teflon into nearby watersheds for many years. The US Environmental Protection Agency claims that this toxin builds up in fish gills and can go far up the food chain (Armand 1994).

Synthetic polymers are a huge problem on land since they are frequently disposed of in landfills, where they will linger for generations while gradually leaching toxins into the soil. This is in addition to their persistence in seas and the water contamination they cause during production. Less than 1% of the synthetic polymer plastic bags that are used each year in the United States, according to the Clean Air Council group, are recycled. In addition to slowly releasing toxic compounds into the soil, the longevity and nonbiodegradability of these synthetic polymers mean that, as their use expands, landfills will continue to be required on a regular basis.

The initial step in the production of conventional petroleum-based synthetic polymers is the distillation of crude oil in an oil refinery. The heavy crude oil is

divided and fractionated into segments or collections of lighter parts. Polymeric hydrocarbon chains with various sizes and structures make up each section. The essential ingredient required to manufacture plastic-making monomers like ethylene, propylene, and styrene is naphtha, one of these fractions. With the aid of particular catalysts, these monomers undergo polyaddition and/or polycondensation to create plastics (Buis 2019; Barker et al. 2007). But this process generates pollutants and greenhouse gases like carbon dioxide (CO₂), which worsens environmental pollution and accelerates global warming (Weiss et al. 2012).

Biopolymers are produced from nature itself, and people believed that since its origin is not toxic. But thorough study is required to understand the effect of biopolymers on the environment. The appeal of bioplastics as alternatives to petroleum-based polymers depends in part on their capacity to achieve both economic and environmental objectives. The following reasons make bioplastics and other bio-based products of great societal, environmental, and commercial interest:

- Their use of renewable resources, which reduces the dependence of plastics on fossil fuels, which are becoming more expensive
- Their potential to provide better recovery and recycling options than petroplastics and other materials, including frequently high biodegradability or compostability
- Their potential to increase industrial competitiveness through novel, eco-efficient bio-based goods and uses
- The potential for bio-based products to create more jobs than biofuels do

Biopolymers and greenhouse gas (GHG) production: GHG production and its effects on climate change are significant aspects of the plastic manufacturing process. It will be extremely difficult for all economic sectors to meet the forecasts for stabilizing atmospheric GHG concentrations at 450 ppm CO₂ by 2050 (Ojeda et al. 2009), which call for emission reductions of 80% from the 1990 levels. Many nations have set goals for such drastic cuts in GHG emissions (Yagub et al. 2014), and many have made developing a national strategy for a bio-based economy a top priority. Based on 44 LCA studies covering roughly 60 different bio-based materials and 350 different life cycle scenarios, a comparison of the cradle-to-grave GHG emissions linked to conventional and bio-based chemicals suggests that the GHG emission savings connected with bio-based products (including bio-based plastics like polytrimethylene terephthalate, polylactic acid, and PHA) are superior to those for their conventional counterparts (Sudhakar et al. 2008). Statements about the advantageous effects of bioplastics for GHG emissions are dubious because of the wide error margins attached to these predictions.

Ocean accumulation of biopolymers: When considering that 75–80 million tonnes of packaging plastics are used globally each year and that 25 million tonnes of plastics are added to the environment each year, much of which ends up in the oceans (Teuten et al. 2007; Jim et al. 2013), it becomes clear that there is a problem with plastic pollution caused by nonbiodegradable materials. The pace of biodegradation is extraordinarily slow once an object is in the ocean. Additionally, there are almost no data on the rate at which plastics mineralize in the marine environment.

While there are often relatively low levels of pollution in ocean waters, hazardous and persistent bioaccumulating pollutants can concentrate when they bind to drifting plastic garbage. The buildup of polychlorinated biphenyls (PCBs), chlorinated insecticides, and polycyclic aromatic hydrocarbons (PAHs), which are proven carcinogens and are thought to be endocrine disruptive substances, on such polymers is of special concern (Naser et al. 2021). Tauten et al. proved that the tendency for dangerous, poisonous contaminants to accumulate on plastics in the marine environment is demonstrated in the laboratory by this (Agrawal et al. 2010).

Fully biodegradable bioplastics do not provide the same issues as traditional plastics that are not biodegradable. However, it has only just come to light that “microplastics,” which can be produced when bioplastics partially degrade, are accumulating in the environment, particularly in coastal habitats (Haider et al. 2019). Microplastics have been proven to be swallowed by a variety of creatures and have enormous surface-to-volume ratios, which may facilitate the exchange of contaminants. If this results in the bioaccumulation of the contaminant load (absorbed and plastic additives), and consequently if microplastics constitute an additional and major vector for transferring pollutants and dangerous compounds, then this is one of the biggest unknowns.

Water pollution: Growing and processing bio-based feedstocks used to make plastics use a lot of water. Industrial agriculture-derived feedstocks have the potential to contaminate water supplies since they used a large amount of chemical fertilizers and pesticides. These may reach to our body through the consumption of polluted water and lead to bioaccumulation of toxic substances (Rosenboom et al. 2022). Current biopolymers put pressure on the available water resources in both of their realities. Several water bodies are contaminated with plastics, which are not intended to degrade in water (Rossi et al. 2015).

Biodegradation of biopolymers: If given the right, controlled environment, biopolymers can biodegrade and recover energy (Naser et al. 2021). Usually, composting is an aerobic process (with oxygen). Compostable biopolymers don’t always improve compost quality or allow for energy recovery (Wang et al. 2021). Not all biopolymers are compostable or biodegradable and will instead be recycled, dumped in the ground, or burned. Composting may provide a few benefits over incineration with heat recovery in municipal solid waste incinerators, according to many research (Wang et al. 2021). Composting can produce a small number of useful byproducts and energy (Wang et al. 2021). CO₂ emissions from municipal incineration and composting are comparable. Although bio-based plastics won’t emit hazardous gases if they don’t degrade, landfilling is the least advised method (Hahladakis et al. 2018). Inconsistent temperatures, a lack of moisture, oxygen, and other factors prevent biopolymers from degrading in a landfill environment (Hahladakis et al. 2018). Polymers that are compostable and biodegradable are made to break down when they are exposed to specific environmental factors. A biopolymer, such as PLA, won’t decompose in a landfill (Hahladakis et al. 2018). This puts more strain on landfills and necessitates extra space for trash. This goes against one of the motivations for biopolymer innovation, which is to decrease garbage generated physically and reliance on landfills. Additives and biodegradable

materials are another issue. Biodegradable plastics that are released into the environment produce microplastics. At any point during their lifespan, bioplastic products have the potential to release additives and microplastics. Conclusions about the costs to the environment and public health associated with the destiny and migration of bio-based microplastics require further study. The potential of biopolymers to recycle help to prevent the carbon cycle and support a circular economy. To reduce the amount of raw materials required and waste should be a top goal to produce valuable byproducts from bio-based resources.

Energy source for biopolymer production: Even though biopolymers originated from natural materials, the processing and purification of biopolymers use non-renewable resource as energy source (Hahladakis et al. [2018](#)).

Conclusion

The health and environmental impact of biopolymers is less when compared to conventional polymers. But it does not mean that they are sustainable in production and completely safe for our environment. There are several factors that determine the synthesis, toxicity, degradation, and features of biopolymers. So each polymer should be studied separately for identifying their impact on the environment. Environmental impact of biopolymers are not only associated with its purified form but the production of raw material and its required resources, processing and purification techniques etc also cause harmful impact on environment. It would prevent the pressure on resources caused by the production of the first-generation feedstocks, which includes the usage of land, water, hazardous chemicals during cultivation, fertilizer input, and emission impacts. The third-generation feedstocks' latent CO₂ emissions into the atmosphere are at their lowest since their carbon is integrated into the polymers. Overall, it would have a lessening effect on aspects of the environment relating to climate change, local ecological harm, and human health. The obvious drawbacks of this generation include its advanced technology needs and energy-intensive requirements. To minimize the negative effects on the environment and human health, responsible integration of sustainable agriculture is required while pursuing the first- and second-generation feedstocks. Despite the fact that bioplastics only occupy a small amount of land globally, it is crucial to utilize that area effectively. The sustainability and efficacy of the feedstock should be taken into consideration when choosing the biomass type for a biopolymer. The first generation of biomass currently has the highest yields and requires the least amount of growing space. Due to their extremely high land efficacy, growers should concentrate on high-yielding crops to reduce the negative effects of intensive agriculture on the environment and human health. Particularly, sugar crops have the most developed infrastructure, the biggest GHG reductions, and the lowest GHG abatement costs followed by starch crops. Because pesticides and fertilizers have such wide-ranging effects on the environment and public health, reducing and regulating their use need to be a top focus. By developing better tools, creating goods with better chemicals, and altering the ways chemicals are utilized, pollution of the soil, water, and air can

be prevented. Each strategy must be developed because of uneven access to any available remedies. By considering these things, we can develop good biopolymers with less harmful effects on humans and the environment.

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Challenges and Issues in Biopolymer Applications

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Mateen Hedar, Azeem Intisar, Tajamal Hussain, Nazim Hussain, and Muhammad Bilal

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Abstract

Biopolymers have recently received tremendous deliberation in numerous daily-life applications. Besides food industry and other industrial applications, biopolymers are mainly employed in biomedical fields for tissue culture and drug delivery. However, these biopolymers offer lower end-use characteristics. As a result, considerable research is currently underway to increase its mechanical properties, crystallization kinetics, thermal resistance, and barrier qualities. Many studies have been carried out in order to improve the qualities of biopolymer to cope with the challenges that reduce their functionalities and applicability. This

M. Hedar · A. Intisar · T. Hussain
School of Chemistry, University of the Punjab, Lahore, Pakistan

N. Hussain
Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan

M. Bilal (✉)
Institute of Chemical Technology and Engineering, Faculty of Chemical Technology, Poznan
University of Technology, Poznan, Poland
e-mail: muhammad.bilal@put.poznan.pl

chapter introduces an overview of various applications of these biopolymers and concludes with a discussion on potential challenges that the group of biomolecules faces in terms of commercial viability.

Keywords

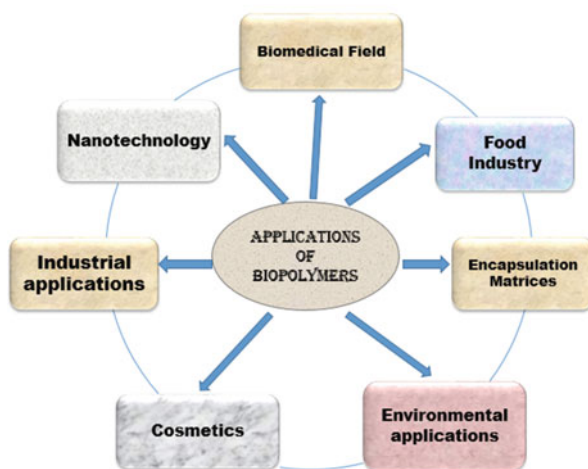
Biopolymers · Biomedical field · Drug delivery · Food industry

Introduction

Biopolymers are derived from natural sources. (Das et al. 2021) Because of their abundance, biocompatibility, and unique properties (Van de Velde and Kiekens 2002) such as nontoxicity (Yadav et al. 2015), biopolymers are fascinating materials. Many of these biopolymers and derivatives have found commercial applications in a variety of industries, including food (Adeyeye et al. 2019) and pharmaceuticals (Jacob et al. 2018). Some of their applications are labelled in Fig. 1. In recent years, the commercial viability of these adaptable material yet durable scaffolds has increased. Biopolymers are being explored for use in a growing number of ways, using different reinforcements to improve their characteristics and practical uses. However, like other materials and methods, use of biopolymers also has pros and cons. The main focus of this chapter is to elaborate issues and challenges using these biomolecules for various applications.

Although biopolymers have numerous advantages, their production has a variety of drawbacks, from beginning their extraction to get their final product. First, because biopolymers are entirely natural, their ultimate qualities are heavily influenced by the raw material. Due to the origin, climate conditions, area, collecting, and handling, this might vary greatly. As a result, global biopolymer

Fig. 1 Applications of biopolymers in various fields of life



manufacturing cannot constantly maintain the same level of sustainability. To date, no universally approved processes for the collection and synthesis of biopolymer material from vegetable sources have been identified. This is critical for both the end product's safety and its quality and functionality. Second, because biopolymer production is still in its beginnings, their production is relatively expensive (Mtibe et al. 2021). Biopolymer manufacturing and extraction from natural and synthetic sources demand specialized tools and instruments that are sometimes not ordinarily available. The expansion of such technology and protocols takes time, money, and qualified staff. Due to the ease and accessibility of biopolymer processing technologies, some existing instruments have been modified to use for this purpose, lowering the expenses in manufacturing of these biopolymers (Ortelli et al. 2019). Their challenges in biomedical and food industry and other drawbacks related to their use are discussed in this review.

Biomedical Fields

Natural biopolymers are advantageous in biomedical applications (Yadav et al. 2015), since they don't produce cytotoxic degradation by-products, and are frequently treated using eco-friendly aqueous-based approaches (Stoppel et al. 2015). Despite the major recent improvements in the pharmaceutical field, several issues and significant tasks such as fine control of biomaterial decomposition rate and spinoffs are restrictive aspects in the translation of tissue engineered products to clinical setups (Kaur and Singh 2020). Although constant research developments in methodology and material blends demonstrate substantial gains in their features that are equivalent to traditional materials now utilized, biopolymers' poorer performance in comparison with traditional materials may decrease their usage (Baloch and Labidi 2021). The usage of biopolymers in gynecology, drug delivery, and wound healing is promising, yet several problems need to be solved.

Challenges of Biopolymer Applications in Gynecology

Even though biopolymers have numerous applications in obstetrics and gynecology (Bibire et al. 2022), they still need to be developed to meet the desired characteristics for their proper use in this field. A few drawbacks have been noted while employing some biopolymers, which vary depending on the type of application and the category of biopolymer used. Many biopolymers decompose quickly and have poor mechanical characteristics which may be undesirable in some gynecological applications (Ostovan et al. 2018). Some researchers advocated employing biopolymer hybrids to solve this problem, and they were able to greatly improve the mechanical characteristics while delaying degradability. Another concern mentioned in the literature is a high hydrophilic capacity, which may not be advantageous in a humid environment like the vaginal canal or in vivo uses (Pattanashetti et al. 2017). Different biopolymer characteristics can be greatly improved to match the requirements of any preferred

application. Even yet, the cost of production may not be efficient or dependable enough for commercialization. It is vital to conduct more upgrades and economic analyses, as well as the development of new materials (Gzyra-Jagiela et al. 2016; Bibire et al. 2022), to solve the cost-effective issue which contribute to scientific knowledge and, as a result, contribute to forthcoming generations and certify sustainability. The key challenges in developing novel biopolymer-based mucoadhesives are examining their long-lasting effect, checking the interactions between the biopolymer and tissues, and bio-dispersion of the formulation content upon vaginal administration (Jummaat et al. 2021).

Challenges of Biopolymer Applications in Drug Delivery

Drug delivery systems are very essential to obtain better therapeutic effects of a medicine at a specific area of disease which may cause less toxicological effect (Jacob et al. 2018). A large number of biopolymers are used in drug delivery (Duttagupta et al. 2015; Saikia et al. 2015). The usage of a drug delivery system permits tailoring of both the rate of drug delivery and the area of drug distribution. It has the potential to overcome systemic drug toxicity, allowing for more efficient disease treatment or tissue remodeling (Abd Rahim et al. 2013; Grøndahl et al. 2020). Several constraints are still preventing the extensive usage of biopolymers. First, most investigations have been carried out *in vitro*; subsequently, additional *in vivo* and clinical trials are prerequisite to determine the well-being advantages of polymers and their biocompatibility in diverse biomedical applications, particularly when utilized as drug delivery encapsulating materials. More research is desired to assess the suitable composite, either unaided or in mixture, to attain the intended payload in an extremely controlled and site-specific way at remedially appropriate amounts when utilized to treat various disorders (Zabihollahi et al. 2020). The improvement of end-use mechanical characteristics, kinetics and release, and heat resistance is a second major constraint. Some products, for example, have a limited mechanical strength, fast deterioration, and a high hydrophilic capacity, making them unsuitable for use in humid or unfavorable environment. Third, in this fast-developing field, difficulties linked to budgets, commercial considerations, and the gap between policy and employment of new methodologies on a worldwide basis must be addressed. The biomaterials have their own biological activities. These materials in conjunction with nanocarriers have synergistic activities. Nanoparticles functionalized by biopolymers have antibacterial activities and are used for drug delivery. However, their toxicological aspect is a major issue. It will be very important to have their detailed study for their effective use in drug delivery (Rai et al. 2015). The biocompatibility of biopolymers for the purpose of drug delivery is important. However, with the lack of rigorous clinical trials, this assertion must be viewed with caution. Just because they come from natural sources and have the same building blocks as the living body does not indicate that they will be able to respond appropriately in a given environment. Effective *in vivo* and clinical testing or well-organized biological models mimicking the application of released medications are required to check compatibility

with living tissue or a living system for toxicity, harmfulness, or physiological reactivity without producing immune rejection (Gopi et al. 2018). Many promising medication candidates fail due to insufficient drug transport to the site of action. Poor medication stability, which can occur at any point of the manufacturing process, as well as during storage and administration, frequently leads to intolerable adverse effects and/or loss of efficacy (Williams et al. 2013).

Silk is a biopolymer which is approved by the Food and Drug Administration. Because of their biocompatibility and biodegradability, it is used in pharmaceuticals as wound dressing (Farokhi et al. 2018). Silk is also widely used for drug delivery (Yucel et al. 2014). Systematic research must be conducted to explore the limitations of silk as a drug delivery system in order for this field to evolve and silk-based products to make a safe progress from bench to bedside. Safe and repeated use of silk for drug delivery still requires more research. Biocompatibility data sets from silk films are insufficient to prove the safety of silk products. In hemato compatibility experiments, we have found that silk has a low thrombogenic reaction and limited hemolytic activity, but its inflammatory response is higher than the clinical reference material polytetrafluoroethylene (PTFE). Silk shows slow thrombogenic response, and its hemolytic action is very low as compared to polytetrafluoroethylene (PTFE) which is a well-known clinical material (Seib et al. 2012). This raises questions about creating unmodified silk for systemic uses until more research is done. Because silk was only recently offered for systemic administration, there are little data to back up the safe transfer of these silk-based drugs to clinics. Examining nanomedicines could be a useful guide for designing and carrying out research that is necessary for this path (Budman et al. 2004). Electrospinning is widely regarded as the most effective nanofiber production technology for biomedical applications. Silk fibroin is used in biomedical field because of simplicity of purification and sterilization. Moreover, it is biodegradable and biocompatible and has lower immunogenicity and higher capabilities to load and stabilize the drugs. These properties, together with the advantages of electrospinning, open up the possibility of developing acceptable silk nanofibers for drug administration. To improve the natural activity of the electrospun silk fibroin nanofibers, several functional moieties can be added. Various methodologies for the production of silk nanofibers have been formulated; however, electrospinning is the most popular because it is a simple and cost-effective method and its processing from different polymeric sources is quite easy (Farokhi et al. 2020). Additionally, nanofibers can be mass-produced on a huge scale for industrial applications (Braghirolli et al. 2014). The production of electrospun silk fibroin and its use in drug delivery are shown in Fig. 2.

Controlled delivery methods are critical in regenerative medicine and chronic illness treatment. These are required to evade drug burst release, which lessens systemic toxicity and unavoidable side effects. This improves therapeutic effects in the targeted area as well. Various polymers can be employed to form diverse delivery carriers. Electrospun silk fibroin mats, for example, are material of choice in drug delivery applications (Wenk et al. 2011). Fabricated silk nanofibers with adaptable diameter and thickness can be made using electrospinning to control drug release kinetics in a specific therapeutic window (Weldon et al. 2012). Despite their

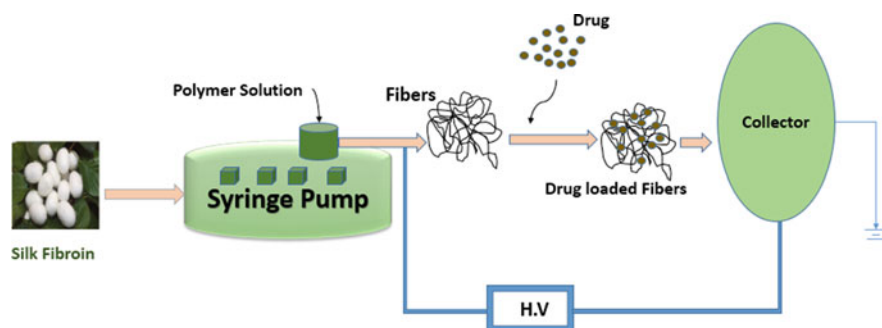


Fig. 2 Electrospun silk fibroin application in drug delivery

advantages as drug delivery platforms, electrospun nanofibers do have significant disadvantages. Although electrospun silk fibers find many applications in drug delivery systems, still it requires further studies to cope with all the problems associated with this technique. One of the key issues is the abrupt discharge of the loaded drug, which could be caused by drug buildup near the nanofibers' surface.

This happened most likely because the drug was mixed in the polymeric solution before the electrospinning process. Another crucial consideration is maintaining the drug's bioactivity during the electrospinning process. Using ultrasonication or high voltage during electrospinning may alter the biomolecule's structure and functionality (Yang et al. 2008). Emulsion and coaxial electrospinning have piqued interest among electrospinning methods because of their capacity to shield medicinal ingredients and reduce the problems of early burst release. Furthermore, drug-polymer contact occurs only during the nanocarrier production phase, significantly reducing undesired reactions between polymeric solutions and loaded biomolecules (Balaji et al. 2015). The properties of silk fibroin sometimes reduce the capability of drug to bind. It has PI 4.2 because of anionic side chains in its strong hydrophilic structure. As pH exceeds than 4.2, their interaction with charged drugs especially positively charged medicines increases. Anyhow, some suitable moieties like SF can be added to modify the product so that it can also interact with anionic drugs (Sagnella et al. 2016). Many studies have confirmed the electrospun silk fibroin mat drug delivery potential in vitro. In vivo trials should be more focused to check the potential of these drug delivery systems. Still, understanding of drug interaction and systematic mechanism of release of drug are not fully known. Another main constraint to use these biopolymers in biomedical field is limited methods of sterilization. Sterilization of these material is difficult because most of the biopolymers are thermally sensitive and their physiochemical properties are effected by heat (Vasconcellos et al. 2012). These treatments can denature proteins and can destruct the loaded drugs (Rnjak-Kovacina et al. 2015). It is very important to use alternative sterilization techniques to maintain the characteristics of biopolymers (Yaman 2001). Batch-to-batch variations in biopolymers also affect their characteristics (J and McClements 2016). Mainly, FDA-approved synthetic polymers are generally used in drug delivery systems (Ghosh 2004). More research is required to use

FDA-approved natural biopolymers in drug delivery. These materials are still under study to use them effectively for drug delivery (Kogan et al. 2007; Singh 2011; Kamaly et al. 2016).

Challenges in the Use of Biopolymers in Wound Dressing

The fundamental role of normal undamaged skin is to permit microorganisms to persist on the surface while preventing their penetration under unbroken skin, which could result in underlying tissue infection or other problems. When the skin barrier is disrupted, infection is significantly more likely since most wounds create an ideal environment for both aerobic and anaerobic bacteria (Bowler et al. 2001). Because of its intricacy, this phenomenon is prone to a variety of anomalies. Several biocatalytic pathways become active during repair and aid tissue healing, in addition to cellular and biochemical components (Sezer and Cevher 2011). Different types of natural and synthetic dressing material are used for healing of wounds. Mostly alginate is considered as an ideal material for wound dressing. It has unique properties like its capability to absorb exudates, and it does not become adhesive to wounds. It can absorb exudate 15–20 times more than its own weight (Smith et al. 2016). It works on the mechanism of ion exchange between dressing and wound (Jones 1999). This mechanism of absorbing the exudate of wound by alginate dressing was studied by Qin who elaborated how Na^+ and Ca^{+2} ions are exchanged during absorbance of exudate (Qin 2004). The mechanism is shown in Fig. 3. Biopolymer-based materials are regarded as smart material. They provide a diverse and tunable framework for designing the proper extracellular matrix to assist tissue

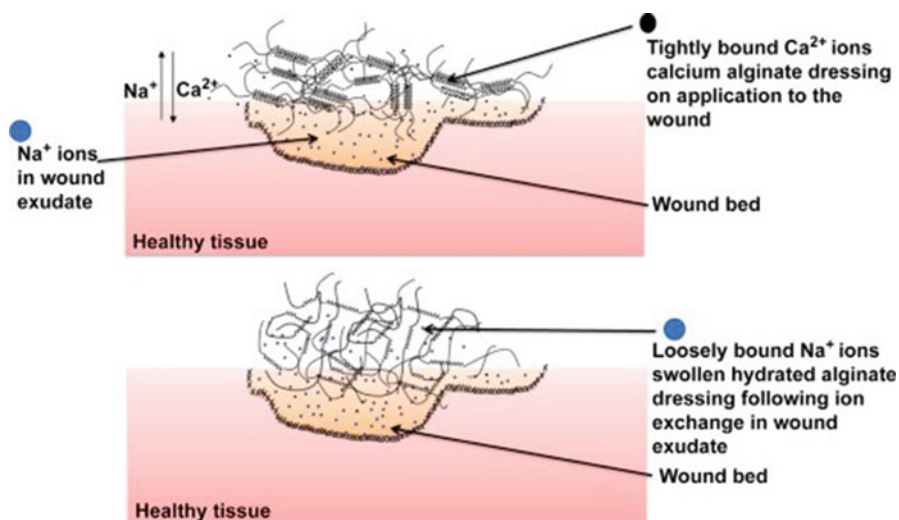


Fig. 3 Mechanism of absorbance of wound exudate by alginate dressing. (Reprinted from (Smith et al. 2016) with the permission of Elsevier)

regeneration while preventing unfavorable events from occurring. Fabrication of bioactive materials based on natural polymers, either protein-derived or polysaccharide-based, has been widely used to address wound-healing-related issues in recent decades (Suarato et al. 2018). However, the use of these biopolymers in pharmaceutical and wound healing has become a challenge to fulfill the requirements of environment-friendly methodologies to meet our future demands.

Environmental sustainability has become an urgent issue to address in a struggle to balance both the industrial production and the earth's ability to produce resources. The main challenges in this regard are proper management to neutralize wastes and to mitigate polluting processes. The healthcare industry's material and energy usage, which ranges from complicated material synthesis processes to medicine packaging to heaps of medical wastes, may contribute significantly to overall effluence, with unintended detrimental consequences for human health (Jameton and Pierce 2001). Several studies have raised concern that alginate material left in the wound can trigger an inflammatory response. However, their biodegradable nature stems from the assumption that biocatalyst present in the body can degrade the trapped fibers of alginate in wound. Berry et al. challenged these claims when he and his colleagues studied the use of Kaltostat for operating cavity wound. Kaltostat is mixture of Na and Ca alginate. They showed the inflammatory reaction in the form of massive cell foreign body reaction (Berry et al. 1996). Scientists have manifested that if these matrices remain in wound, they can cause inflammatory reaction. Therefore, professionals should be cautious when removing alginate dressing, like any other dressing, and make sure it is done with extreme care (Thomas 1992). Furthermore, the development of genomics and proteomics studies has enhanced the demand for more individualized medicines that are developed based on the understanding of the unique biology of the patient. The various beneficial features of biopolymers are expected to be used in creating and delivering these cures in the future, and this has the potential to transform wound remedial treatments (Smith et al. 2016).

Food Industry

Biopolymers are extensively used in food items (Stoica et al. 2020). Biopolymers are used in food as well as in food packaging (Khan et al. 2007). Various biodegradable polymers like carbohydrate, starch, and lipids are frequently used to make food material. Yeast and other products obtained from bacteria, fungi, and algae are used in food products. The techniques used for the production of these materials are cost-effective and easy and require few steps and do not pollute the environment (Grujic et al. 2017). The edible biopolymers can cause no or very minute hazards as they are biodegradable and meet the standards of edible products (Baranwal et al. 2022). However, the material used in the oxygen barrier food packaging sometimes faces some challenges and issues (Gheorghita Puscaselu et al. 2021). Mostly, biopolymers are used as an emulsifying agent to stabilize the food emulsions and to improve their physical and chemical properties. However, it is a big task to choose the most suitable emulsifier because they differ considerably in their capability to stabilize

food emulsions and their ability to perform (McClements 2009). In order to prevent any bad impacts that may occur with these innovative materials, it is obvious that the scenario requires a hazard analysis and vulnerability assessment to be conducted.

Challenges in the Use of Biopolymers for Food Packaging

A majority of the materials used to pack food are nonbiodegradable and cause environmental hazards. For a decade, the utilization of biopolymers as a smart agent for the packaging of food products has drawn a lot of attention from biochemists, and it has become an emerging field to use these bio-products for the safety of food. These polymers are effectively used to pack food and to monitor the quality of food. The entrapment of micronutrient in biopolymers and antioxidant packing of food have made the food safety easy. Moreover, the use of smart and intelligent packing mechanism has the potential to enhance the shelf life of edible items (Stefani et al. 2016). Different types of polymers are utilized to produce environment-friendly food packing. However, the use of these materials in food packing has to face various feasibility issues which include their high budget for product, and this expensive process also does not give a better performance in comparison with their counterparts – synthetic polymer products – employed for food packaging. Therefore, the use of this method at industrial level still finds its limitation. Scientists are trying to introduce a method for their cost-effective production and use in packaging. Biopolymer-associated nanocarrier can provide the smart characteristics to food packing. These nanocomposites can add antibacterial, oxygen scavenger ability and biocatalyst immobilization. It can also indicate the decomposition-related factors. However, safety concerns are also associated with the use of these nanocomposites in packaging, as the size of nanocomposite is very small and they can easily get into the cell by penetration. The qualities and safety of biopolymer material used in bulk for the formation of nanocomposite is typically understood, but the nanocarriers associated with them frequently display unique properties as compared to biopolymers which can also cause health hazards. The scientific research on the migration of these materials from food packing directly into food and indirectly to the human body is scarce. But there is a plausible possibility that they can get into the food. Therefore, more studies are needed to get the precise information about their migration to avoid health hazards after their prolonged exposure (De Azeredo 2009). The use of cellulose- and starch-based products does not cause any issues to health, and these biopolymers are eco-friendly (El-Saied et al. 2004; Medina-Jaramillo et al. 2017). However, the biopolymers like polyhydroxyalkanoates (PHAs) and polylactic acid obtained from different microbes cause problems when used in food packaging. The strains of microbes which are used to extract these materials could be possibly precarious (Conn et al. 1995). In contrast to the additives employed in the fabrication of traditional plastics, biopolymer materials typically use different types of additives. The potential passage rate of biopolymers' constituents depends on their physical and chemical characteristics and the environment in which they are produced. The qualities of clean biopolymers

(those without additives) are typically less than those of traditional ones. The use of starch-based nanocomposites in food packing does not cause any environmental and health issue (Avella et al. 2005). Oxo-biodegradable materials are the most prevalent cause of migration of chemicals from food packing into the food (Grujić et al. 2017). Although, the nano-biocomposite showed cytotoxicity effects however, it is not possible to attain recognized uniform testing conditions because of production of incredible diversity of material even prepared in a same batch. These investigations are not able to take into account the final decision about their toxic capability (Jorda-Beneyto et al. 2014).

Challenges in the Use of Biopolymers for Industrial and Environmental Applications

The key factors influencing the prevalent approval of natural products and biopolymers as green matrix are the quick depletion of petroleum resources and the growing attentiveness to environmental issues associated with the usage of traditional plastics. Because of their sustainability and eco-friendly nature, these products have garnered a significant interest from scientists and industrialists. They can be used for various applications in textile, food, automotive, and bioenergy production (Sanyang et al. 2016). Sugar palm is a multiuse plant, and it is highly enriched with natural products and biopolymers which can be extracted and used for various industrial applications (Huzaifah et al. 2017). Sugar palm fibers (SPF) largely contain cellulose which is recognized by its extraordinary mechanical properties. The starch obtained from sugar palm plant can be used for plasticization, or it can be blended with other biopolymers to reinforce its characteristics to find their potential applications at industrial level. Significant work has been done on sugar palm-based composites thus far, but no published research on sugar palm-based nanocomposites has been done. Investing in sugar palm nanocomposites can help the industry's image and offer up new markets, including those for electronic and pharmacological packaging. In order to solve various issues impeding future industrial applications of sugar palm fibers, biopolymers, and their composites, an innovating and virgin research is required for this brand-new area of study and invention (Sanyang et al. 2016). Moreover, further studies are required to use these biopolymers at a large scale, because their manufacturing at a large scale and the difference in the batch-to-batch products might cause hindrances in their proper use (Imre and Pukánszky 2013; Sanyang et al. 2018). Ford was the earliest automaker to use biopolymer-containing plastics in paints, varnishes, and parts made of molded plastic (Drzal et al. 2001; Brosius 2006). Additionally, Ford used natural fibers in a variety of the car's components (Phillips 2008). The biopolymers are mostly used for 3D printing and find promising application in various industrial fields. The additive manufacturing (AM) of biopolymers consists of a series of steps that change the computer-aided design (CAD) into composite or other designs in a layered fashion (Wong and Hernandez 2012; Gibson et al. 2021). 3D printing is more advantageous as compared to conventional methods. However, the waste products of this processing

cause a threat to the environment. Heaps of garbage and plastics end up in seas and open places, which is also hazardous for human beings as well as other animals. The use of biopolymers and biodegradable materials in this process is more beneficial because of their eco-friendly properties. The required material qualities also differ greatly depending on the intended use. For instance, a material must have a holistic characteristic that includes printability, biocompatibility, degradability, and mechanical qualities in order to be used in the biomedical industry. Contrarily, textile items need to be sufficiently flexible, resilient, and tensile in order to accommodate the wearer's comfort and movement. Additionally, edible, easily printable, and resistant to shape distortion during post-processing are all desirable qualities for 3D-printed foodstuffs (Liu et al. 2019, Li et al. 2021). For example, a novel cellulose approach was used for the modification and functionalization of textiles. Extensive studies on the adsorption of the cellulose derivatives to cellulose and durability testing were integrated with the prototyping of 3D-printable cellulosic textile applications utilizing a design-driven technology. The QCM-D peeling and washing tests proved that acetoxypentyl cellulose (APC) was not very firmly bonded to cellulosic materials because of its more branched molecular structure. The printed structures had strong adsorption and great linkage qualities as a result of the rectilinear structure of cellulose acetate (CA), which allowed the material to arrange in a line with the cellulose molecules in the substrate. Both materials may be used in fabric 3D printing. The process of 3D printing is illustrated in Fig. 4.

Although many well-designed and graphical textile demonstration prototypes were effective in showcasing their potential for a wide range of application possibilities, the use of 3D-printed cellulosic components on cellulose fabrics opens up new possibilities for the development of customizable all-cellulose textiles without labor-intensive processing. Additionally, the creation of new materials and services could be done in an environmentally sustainable way by utilizing recyclable and renewable resources (Tenhunen et al. 2018). The main concern in the use of biopolymers in 3D processing is strength and inflexibility of these products. Their mechanical characteristics are not comparable to the conventionally used products. Therefore, there is a restriction in the applicability of biopolymers in 3D printing to an extent. Moreover, biopolymers are not thermostable. They decompose in high temperature, while in 3D printing process, materials having a high thermostability

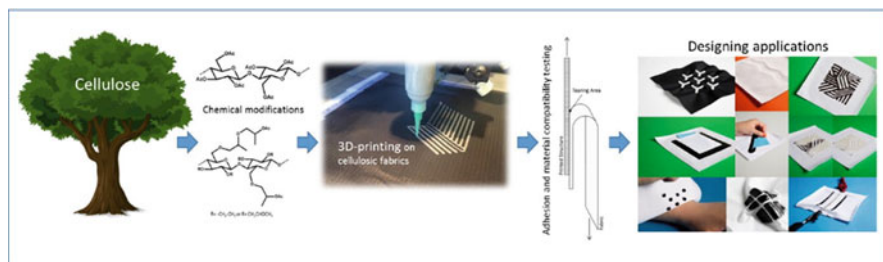


Fig. 4 The use of cellulose and its derivative for 3D printing in textiles. (Reprinted from (Tenhunen et al. 2018) with the permissions of Elsevier)

can broaden their applications. More research and advances in 3D printing technology are required. An efficacious development and marketing of these material can also be made possible by advancements in engineering technology and 3D printing techniques (Pradhan et al. 2021).

Future Prospective

Exploring alternate methods for the manufacturing of bioplastics and recyclable polymers has become possible as a result of emergent environmental apprehensions regarding traditional plastic materials. By overcoming the difficulties, the possibility of employing biopolymers can become a reality. They should be adapted and modified for commercial applications. The creation of custom biopolymer structures is necessary because pure polymers cannot provide the desired characteristics to final the product for their applicability at industrial level. Moreover, various post-processing methods should be changed, and more advancement is required to improve the performance in order to keep the actual shape of the product. In addition to chemically modified biopolymers, the physically blended biopolymers can provide another method of producing products with advantageous processability, printability, mechanism, and bioactivities properties. More research is required to fill the knowledge gap to overcome the challenges and issues in biopolymer applications so that new ways can be paved for new technology and advancement in the previous ones to achieve an effective production and applicability of these products.

Conclusion

Biopolymers are extensively utilized in food, industrial, and various other fields of life. Due to their distinctive qualities, including low density, easy manufacturing, and chemical stability, polymers are appealing for a variety of applications. The biopolymers used in conjunction with other materials like nanoparticles to form nanocomposite for use in food packaging can cause a major concern to food safety because of the migration of these nanomaterials into the food. It is necessary to determine the maximum nanoparticle concentrations that can be present in food. The biggest problem researchers are facing in recent times appears to be establishing such regulatory restrictions, as well as their limited thermostability and mechanical characteristics. Research is still required to understand the mechanism by which biopolymers can be used for proper food safety and in environmental, industrial, drug delivery, and pharmaceutical fields, as well as their influences on the human body and the ecosystem. Beyond these challenges, these biopolymers provide a chance to increase the range of applicability of raw materials, which is not possible with the utilization of synthetic polymers. Because of the many benefits that biopolymers offer, this objective can be achieved. Biopolymers can be used as alternatives to avoid the disposal of huge amounts of garbage that nonbiodegradable packaging materials produce. There are various obstacles to the industrial synthesis

of biopolymers that have not been investigated yet to determine their viability from an economic and environmental standpoint. For their applicability and functionality to evolve more quickly and effectively, a comprehensive examination and a detailed study and trial of these biopolymers are necessary to overcome the challenges and issues associated with them.

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